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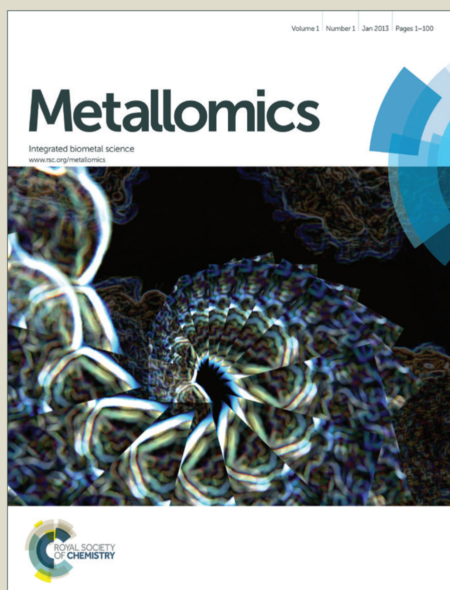

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Iron transport in the kidney: Implications for physiology and cadmium nephrotoxicity

Frank Thévenod[1]* & Natascha A. Wolff[1]

[1]Institute of Physiology, Pathophysiology & Toxicology, Center for Biomedical Training and Research (ZBAF), Stockumer Str. 12, University of Witten/Herdecke, 58453 Witten, Germany

frank.thevenod@uni-wh.de

natascha.wolff@uni-wh.de

*Corresponding author

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1. Abstract

The kidney has recently emerged as an organ with a significant role in systemic iron (Fe) homeostasis. Substantial amounts of Fe are filtered by the kidney, which have to be reabsorbed to prevent Fe deficiency. Accordingly Fe transporters and receptors for protein-bound Fe are expressed in the nephron that may also function as entry pathways for toxic metals, such as cadmium (Cd), by way of "ionic and molecular mimicry". Similarities, but also differences in handling of Cd by these transport routes offer rationales for the propensity of the kidney to develop Cd toxicity. This critical review provides a comprehensive update on Fe transport by the kidney and its relevance for physiology and Cd nephrotoxicity. Based on quantitative considerations, we have also estimated the *in vivo* relevance of the described transport pathways for physiology and toxicology. Under physiological conditions all segments of the kidney tubules are likely to utilize Fe for cellular Fe requiring processes for metabolic purposes and to contribute to reabsorption of free and bound forms of Fe into the circulation. But Cd entering tubule cells disrupts metabolic pathways and is unable to exit. Furthermore, our quantitative analyses contest established models linking chronic Cd nephrotoxicity to proximal tubular uptake of metallothionein-bound Cd. Hence, Fe transport by the kidney may be beneficial by preventing losses from the body. But increased uptake of Fe or Cd that cannot exit tubule cells may lead to kidney injury, as well as Fe deficiency that may facilitate renal Cd uptake.

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2. Introduction

Until recently, the kidney was thought to play no role in systemic iron (Fe) homeostasis [1]. Interestingly, cadmium (Cd) has been known for decades to accumulate in the kidney (described in details in the excellent review By G.F. Nordberg [2]). The major Fe compounds in biological systems are the redox pair ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron whereas the major Cd compound is the divalent Cd ion (Cd^{2+}). Due to their hydrophilicity Fe^{2+}/Fe^{3+} and Cd^{2+} (and other metal ions) must cross cellular membranes via proteinous pathways, i.e. channels, transporters or receptors. In biological systems Fe^{2+}/Fe^{3+} and Cd^{2+} are mostly found in bound form and are either complexed to small ligands, such as amino acids or peptides [3], or more or less specifically bound to proteins (e.g. ferritin, transferrin, metallothionein as opposed to albumin) whose affinity constants determine their residency as "free" or bound metal ions. As a non-essential metal in mammals, Cd^{2+} must exploit and compete for physiological entry pathways for essential metal ions, such as Fe^{2+}, Cu^{2+}, Zn^{2+} or Mn^{2+}. To describe this process, the term "ionic and molecular mimicry" has been coined [4]. In this context, molecular mimicry accounts for a condition in which a toxic metal ion forms a complex with an endogenous organic ligand (e.g. a peptide or a protein) and the resulting compound mimics the behavior of the endogenous ligand that binds to its receptor.

Interestingly, epidemiological studies had shown an inverse relationship between the size of the Fe stores and the Cd burden of the body (and kidneys) [5] [6], thus hinting at a link between transport of Fe^{2+}/Fe^{3+} and Cd^{2+} (reviewed in [7]). Mounting functional evidence for transport of Cd^{2+} by transporters and receptors for essential free and complexed metal ions in renal and other epithelia [8] [9] was then superseded by the discovery that the first cloned Fe^{2+} transporter, the divalent metal transporter 1 (DMT1/Nramp2/DCT1/SLC11A2), is equally well permeated by Cd^{2+} [10] [11]. DMT1 (and other Fe^{2+}/Cd^{2+} transporting proteins) is, however, highly expressed in the kidney.

This fact soon attracted the attention of Craig P. Smith from the University of Manchester (U.K.) who noticed that the kidneys *"…contain many if not all of the proteins that are central to iron balance, that in some cases are expressed in considerable amounts, implies that the kidney handles iron in some way that has demanded evolutionary conservation and therefore is likely to be of*

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importance…. [12]. In a series of pioneering studies, his group measured Fe reabsorption by the rat kidney *in vivo* [13]. From their data they estimated that under physiological conditions ~0.4 mg Fe is filtered daily by rat kidneys, but only 0.7% is excreted in the urine and also appears to depend on the renal expression of DMT1 [14 15]. Alterations of dietary Fe intake modulated renal DMT1 expression: Iron restriction increased renal DMT1 whereas iron loading decreased renal DMT1 expression and DMT1 expression was inversely correlated with urinary Fe output [15] and therefore they concluded that long-term modulation of renal DMT1 expression may influence renal iron excretion rate. In addition, it soon became apparent that a certain proportion of filtered Fe is protein-bound and includes transferrin (Tf) [16 17]. Meanwhile, additional renal Fe transport pathways have been identified and characterized that now allow a better understanding of the role of the kidney in Fe handling and physiological Fe homeostasis. Thus, the notion that the kidney is involved in transport and excretion of Fe and other metal ions [12] has gained recognition and has entered the fields of toxicology [18], iron biology [19 20] and nephrology [21].

3. Systemic Iron homeostasis

For detailed accounts of systemic Fe homeostasis, the reader is referred to excellent recent reviews [1 22 23] . Iron, with an amount of ~2.5-4.5 g in adults, is the major transition metal in the body and is mostly localized in erythrocyte hemoglobin, amounting to roughly 60% of the human total body Fe. Yet, Fe is indispensable for other tissues as well, being an essential component of hundreds of proteins, including many enzymes. Thus, Fe is not only required for oxygen transport and storage with hemoglobin or myoglobin, Fe-containing proteins are needed for a variety of additional functions, including, first and foremost, mitochondrial respiration (electron transport chain), but also metabolism and detoxification (cytochrome P450 enzymes), DNA synthesis (ribonucleotide reductase), antioxidant defense (catalase) and beneficial pro-oxidant functions, oxygen sensing (hypoxia-inducible factor (HIF) prolyl hydroxylases), and immune defense (myeloperoxidase) [24]. Yet, Fe is also toxic due to the production of cell-damaging radicals through *Fenton*-type reactions [25] Thus, body Fe homeostasis needs to be tightly regulated.

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Mammalian Fe homeostasis is unusual in that it is mainly controlled at the level of intestinal Fe absorption. To date, there is no known regulated short-term mechanism of Fe excretion (but see section *2.* for an example of long-term modulation in the kidney; reviewed in [12]) and the small daily Fe loss of about 1mg in healthy adult males is closely balanced by duodenal Fe uptake. The daily Fe loss mainly (~80%) occurs via shedding of Fe-laden duodenal enterocytes, complemented by much smaller losses via other pathways, including the kidneys [26] (reviewed in [27, 28]). The large fraction of Fe in erythrocyte hemoglobin is efficiently recycled, while excess Fe is stored in the liver (0.5-1g) (reviewed in [27, 28]). Under normal conditions, Fe loss is balanced via regulated intestinal absorption [29]. Two forms of dietary Fe are taken up into duodenal enterocytes via different mechanisms: While heme Fe uptake occurs via not yet clearly defined pathways (see [30, 31, 32]), non-heme Fe absorption is mediated by the proton-coupled divalent metal transporter 1 (DMT1/Nramp2/DCT1/SLC11A2) [10] (reviewed in [33]) after reduction of dietary Fe^{3+} to Fe^{2+} by duodenal cytochrome B with ascorbate as an electron donor (reviewed in [34]). An apical intestinal Na^{+}/H^{+} exchanger appears to be responsible for generating the proton gradient necessary for DMT1-mediated Fe^{2+} uptake [35]. Fe^{2+} is subsequently delivered to cytoplasmic ferritin for storage [36] by chaperones, including the poly (rC)-binding protein 1 (PCBP1) [37], or to the basolateral transporter ferroportin (FPN1/IREG1/MTP1/SLC40A1) [38, 39, 40, 41] for efflux into the plasma. Efficient export requires the presence of members of a family of copper-containing ferroxidases, e.g. hephaestin and/or ceruloplasmin [42, 43, 44], which convert effluxed Fe^{2+} to Fe^{3+} that mainly binds to the Fe-carrying serum protein Tf. Importantly, FPN1, to date the only known cellular Fe exporter [45], is regulated by Fe loading [46] through homeostatically increased synthesis and release of the hepatic peptide hepcidin into the circulation that limits further absorption of dietary Fe and its release from stores (reviewed in [47]): Hepcidin binds to FPN1, leading to its internalization and subsequent lysosomal degradation, hence preventing further Fe export into the plasma [48].

Free, unbound Fe is incompatible with either plasma Fe transport (it would precipitate) or with cytosolic Fe trafficking (it would damage the cellular environment) [49]. Therefore, Fe must be complexed with appropriate ligands. The transport of Fe in plasma to its sites of use occurs predominantly as Tf-bound Fe [50] (TBI), and to a lesser extent associated with several other serum

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proteins, including ferritin, albumin, neutrophil gelatinase associated lipocalin (NGAL/24p3/lipocalin-2), and possibly lactoferrin and hepcidin. Collectively, these latter forms of serum Fe - with the exception of ferritin - are termed non-Tf-bound Fe (NTBI) [51 52 49]. Under physiological conditions, NTBI is a minor entity within total serum Fe - although NTBI may become a relevant issue in patients with various pathological conditions in which Tf saturation is significantly elevated (reviewed in [49]). Ferritin, primarily an intracellular protein, is low in human serum under normal conditions, despite substantial inter-individual variations and substantial increases under Fe overload conditions [53] where it may be secreted through a non-classical lysosomal secretory pathway by macrophages and renal proximal tubule (PT) cells [54]. Additionally, serum Fe may exist in the form of holo-Tf bound to a soluble form of the Tf receptor [55].

4. Cellular iron homeostasis

One major mechanism for Fe assimilation by erythrocyte precursors and non-erythroid cells is the internalization of serum Tf-bound Fe^{3+} [56 57]. Tf endocytosis is mediated by the ubiquitous Tf receptor 1 (TfR1) [58 59] (a TfR2 has been cloned but its expression is limited to the liver and erythropoietic progenitors [60] where it is thought to operate as an “Fe sensor” [61]). Endosomal acidification favors the release of iron from Tf, which itself remains bound to the receptor and is subsequently recycled to the cell surface, where the near neutral pH promotes dissociation of apo-Tf from the receptor and its release into the circulation [62 63]. Endosomal Fe^{3+} is quickly reduced to Fe^{2+} by an oxidoreductase activity, now known to be represented by “Steap” (sixtransmembrane epithelial antigen of the prostate) family proteins, namely Steap2 to Steap4 [64 65], (a reaction which may actually occur prior to dissociation from the Tf-TfR1 complex, especially since Fe^{3+} tightly binds to Tf, while Fe^{2+} does so only weakly [66]). Subsequent endosomal efflux of Fe^{2+} is mediated by DMT1 [67 68]. The transient receptor potential mucolipin 1 (TRPML1/ML1/MLN1/MCLN1) may function as another Fe^{2+} release channel in late endosomes and lysosomes [69].

The mechanisms of intracellular Fe trafficking to its sites of utilization are not well understood. In most cell types, it is agreed that Fe acquired during the Tf cycle is first released into the cytosol by

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entering a "labile cytosolic Fe pool" that is defined as a pool of chelatable and redox-active Fe^{2+} and represents a transition compartment for Fe sensing, metabolic utilization or storage [70 71]. A variety of low molecular weight compounds have been suggested as Fe chelators in this readily accessible Fe reservoir, including organic anions like citrate and phosphate, oligopeptides such as glutathione (GSH) [72], membrane phospholipids, as well as "mammalian siderophores", namely 2,5-dihydroxybenzoic acid (2,5-DHBA) [73], although an involvement of the latter has been recently challenged [74]. Further, the conserved cytosolic glutaredoxins Grx3 and Grx4 could also play an essential role in intracellular Fe sensing and trafficking, as their depletion in yeast leads to impaired Fe transport to mitochondria and defects in Fe-dependent pathways [75].

In contrast, in erythroid cells, kinetic and microscopy studies support a "kiss and run" hypothesis, which assumes the direct delivery of Tf-derived Fe to mitochondria through a transient contact with endosomes (reviewed in [76]): This concept was originally developed based on kinetic studies with ^{59}Fe-Tf in reticulocytes at 4°C that contain very little chelatable cytosolic Fe, thus preventing Fe mobilization from other compartments [77]. Ponka and coworkers later observed direct, albeit transient inter-organellar contacts and a simultaneous increase in mitochochondrial chelatable Fe at these sites by live confocal imaging [78].

The major Fe-utilizing cellular organelles are mitochondria that require Fe for the synthesis of heme and Fe–sulfur clusters [79 80]. Irrespective of whether Fe is delivered by cytosolic chaperones or direct endosome-mitochondria contacts, it has to cross two membranes to enter the mitochondrial matrix where it is needed for synthetic processes. The outer mitochondrial membrane (OMM) has typically been assumed to be freely permeable to Fe due to the presence of "pores" [81] represented by voltage-dependent anion channels (VDACs) that are regarded as the major permeability pathway of the OMM for small solutes [82]. But this knowledge is based on *in vitro* studies that have been performed after reconstitution of VDAC in artificial membranes/planar lipid bilayers. Thus, *in vivo* the OMM may not be as freely permeable to inorganic cations as previously believed and VDAC function may be tightly regulated [83 84]. We have recently identified DMT1 in several tissues as a possible

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mechanism for Fe^{2+} transfer across the OMM using a variety of experimental approaches [85] [86] (see section *7.1.3.*), but additional pathways may also exist.

Entry of Fe into the mitochondrial matrix requires the SLC transporter mitoferrin-1 (also known as MFRN1/SLC25A37), which is found in the inner mitochondrial membrane (IMM) [87]. Mitoferrin-1 is highly enriched in erythroid cells and is stabilized during differentiation whereas mitoferrin-2 is ubiquitously expressed and its half-life is not regulated [88]. The lack of functional mitoferrin-1 in the *frascati* zebrafish mutant is associated with severe defects in erythropoiesis, heme synthesis and Fe-sulfur clusters biogenesis [87]. Some studies have also suggested that the mitochondrial calcium uniporter in the IMM represents an additional route of Fe entry into the matrix (e.g. [89]).

Cells may eliminate excess intracellular Fe by secretion of Fe^{2+} via FPN1 or by secretion of heme through the putative heme exporter FLVCR (feline leukemia virus, subgroup C, receptor) [90]. Excess intracellular Fe may also be stored and detoxified in the cytosol by ferritin, which consists of 24 H (heavy) and L (light) subunits, encoded by two different genes [91]. H-ferritin possesses ferroxidase activity, mediating conversion of ferrous Fe (Fe^{2+}) to the ferric form (Fe^{3+}), whereas L-ferritin chains provide a nucleation center. Ferritin assembles into a shell-like structure with a cavity of ~80 nm that provides storage space for up to 4500 Fe^{3+} ions. Shuttling of Fe to ferritin appears to be mediated by the PCBP family chaperones [37] [92] (see section *3.*). Both the lysosomal and the proteasomal pathways of degradation seem to be recruited to mobilize Fe from ferritin, probably depending on the cell type and the cellular conditions (reviewed in detail in [91]). Mitochondria contain a nuclear-encoded ferritin isoform [93] whose expression is limited to a few organs, such as testis, neurons, heart and kidney, but not the liver or spleen [94]. Mitochondrial ferritin may cooperate with cytosolic ferritin in the maintenance of intracellular Fe balance or protect mitochondria from Fe-dependent oxidative damage and increased production of reactive oxygen species (ROS) in cells with high metabolic rate [91].

5. Function of the kidney

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Detailed state-of-the-art descriptions of the morphology of the kidneys, the structure of the nephrons, i.e. the functional units of the kidneys, and their functions are beyond the scope of this review and can be found in standard handbooks of renal physiology [95] [96] [97]. The aim of this very simple overview on kidney function is to introduce readers unfamiliar with renal physiology to basic principles that are required for a better understanding of handling of Fe and Cd by the kidneys.

The blood volume permanently equilibrates with the interstitial fluid of the extracellular space and - through the interstitial fluid - with the intracellular space. Together with the lungs and the intestine, the kidney keeps the body fluid homeostasis of mammalian organisms constant by selectively excreting metabolic wastes, excess solutes and water as well as xenobiotics from the body into the urine. Blood is constantly pumped through the kidneys where plasma fluid is filtered through a capillary network called the glomerulus. The driving force for ultrafiltration is generated by the effective filtration pressure in the capillaries, which is set by the glomerular blood pressure. To fulfill the excretory function of the kidneys, large quantities of plasma amounting to >60x its total body volume are filtered daily in the renal glomeruli, complemented by secretory pathways along the renal tubule epithelium. Filtered water and solutes still of use for the body are efficiently recycled to the circulation by obligatory and regulated reabsorptive processes in the tubular sections of the nephrons. By these means, about 180 l of primary filtrate is generated every day to produce about 1-3 l final urine. This indicates that about 99% of the primary urine is reabsorbed along the more than 2 million nephrons.

At the glomerulus, a three-layer anatomical barrier allows fluid and solutes <10kDa and/or 18Å to cross that barrier, but permeation decreases with increasing molecular mass (cutoff of ~80kDa), molecular size (<42Å), and also depends on charge (cationic>neutral>anionic) (see however [98] for a critical discussion). Hence, the primary urine also contains essential nutrients and electrolytes that need to be actively reabsorbed to avoid critical losses and ensuing deficiencies. On the other hand, some metabolic wastes are actively secreted by the kidney since their rate of production exceeds their rate of glomerular filtration. All these selective processes are carried out by the nephrons, epithelial tubular structures that consist of several interconnected segments with characteristic

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morphological and functional properties, the PT with its convoluted segments S1 and S2 (PCT) and straight segment S3, the loop of Henle (LOH), the distal tubule (DT) with its convoluted segment (DCT) and connecting tubule, and finally the collecting duct (CD) (see Figure 1). Glomeruli, convoluted segments of the PT, DT with connecting tubule, and cortical CD are localized in the kidney cortex. Parts of the straight segment of the PT, parts of the thin limb of the LOH, the thick ascending limb of the LOH and outer medullary CD are in the outer medulla. The remaining segments (most of the thin limbs of the LOH as well as initial and terminal inner medullary CD) are found in the inner medulla of the kidney.

In general, the PT is responsible for bulk reabsorption of the primary fluid that is filtrated in the lumen of that segment. About two-third of PT reabsorption occurs “paracellularly” at intercellular tight-junctions, through osmotically driven “solvent drag”. But amino acids, glucose, bicarbonate and several other essential molecules are also reabsorbed via luminal Na^+-dependent transporters expressed in the luminal brush-border membranes (BBM) of PT cells and therefore require the energy of adenosine triphosphate (ATP) for activation of basolateral Na^+/K^+-ATPases to maintain these reabsorptive processes. The PT cells are also responsible for bulk reuptake of filtered proteins and peptides via a multi-ligand receptor complex expressed in the luminal BBM, megalin:cubilin:amnionless [99] (see section *7.1.1.*), that also binds metalloproteins, such as Tf (an Fe binding protein) or metallothionein (MT) (a Cd^{2+} binding protein) [100]. Finally, the PT is the major location for the secretion of xenobiotic and endogenous organic cations and anions. The LOH that follows the PT builds up the hyperosmotic interstitium surrounding the final segment of the nephron, the CD that is required for reabsorption of water to generate small volumes of concentrated urine (“antidiuresis”), thus preserving water for the body. Hyper-osmolarity of the kidney medulla is built up by several properties of the different segments of LOH, i.e. 1) active NaCl transport into the interstitium by the thick ascending limb of LOH; 2) high permeability of the descending LOH to water and low permeability of the ascending LOH; 3) increased permeability to urea in the medullary portions of LOH; and 4) magnification of the medullary hyper-osmolarity by the countercurrent flow within the descending and ascending limbs of LOH (“countercurrent multiplication”) [95 96 97]. A hypo-

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osmotic fluid reaches the DT where divalent metal ions are reabsorbed, such as Ca^{2+} and Mg^{2+} (and Fe^{2+}) (see section *7.3.*) and the luminal fluid is further depleted by active NaCl reabsorption. In the CD the final composition of the urine is adjusted by "fine-tuning" through hormonal regulation of the CD cells. The paracellular permeability of the CD epithelial layer to ions and water is very low; therefore the final content of the urine in NaCl and water must be controlled by hormonal regulation of CD cells via aldosterone and antidiuretic hormone. This occurs through regulated and temporary incorporation of epithelial Na^{+} channels (ENaC) and aquaporin-2 water channels (AQP2), respectively, into the apical membrane of principal (light) cells of the CD. Additional regulated functions of the CD include acid-base balance (type A- and type B-intercalated cells) and K^{+} homeostasis. Apart from the CD, PT and DT also represent nephron segments where hormones (i.e. parathyroid hormone, calcitonin, calcitriol) control Ca^{2+} and PO_4^{3-} homeostasis. Figure 1 summarizes the structure of the nephron with the major functions of the different nephron segments that are relevant to this review.

6. Plasma iron and renal glomerular filtration

Only recently has it been recognized that the kidney is also involved in systemic Fe homeostasis because certain Fe-containing complexes in plasma (e.g. Tf, NGAL/24p3/lipocalin-2, lactoferrin, albumin, hemoglobin, myoglobin and hepcidin) have the ability to cross the glomerular filter, even under physiological conditions [101 102] (reviewed in [103 12]). There is also a rising interest as well in the role of Fe in both acute kidney injury and chronic kidney disease [21]. Renal Fe losses are minimal under physiological conditions [26] (reviewed in [27]). The lack of urinary Fe excretion has traditionally been attributed to binding of Fe (or, if erythrocytes are lysed within the blood, hemoglobin and free heme) to larger proteins that would ensure that little or no Fe is lost by glomerular filtration and entry into the urine because of the low protein permeability of the glomerular filter [104 28]. That would also include the large 24-subunit serum ferritin complex that is unlikely to reach the ultrafiltrate. But NGAL is present in plasma as monomers of 25 kDa and dimers of 45 kDa that should easily permeate the glomerular filter [105]. Similarly, the small molecule hepcidin (2-3 kDa) readily passes into the primary urine [47]. Moreover, early micropuncture studies in animals indicated significant glomerular

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filtration of high-molecular weight proteins (HMWP) , such as albumin (reviewed in [106 98]). In accordance with these observations, patients with renal *Fanconi* syndrome, i.e. with compromised renal PT function, including protein reabsorption (reviewed in [107]), display increased urinary excretion of proteins up to 160 kDa [17], suggesting that substantial amounts of TBI, i.e. Fe bound to Tf (80kDa), as well as NTBI (see section *3*), such as Fe bound to albumin (66.5 kDa) and lactoferrin (80 kDa), reach the primary filtrate and must be reabsorbed by the PT (see *7.1.1.*).

7. Iron transporters of the nephron

(see also Tables 1 and 2 for a summary).

7.1. Iron transporters of the proximal tubule (PT)

7.1.1. Megalin:cubilin:amnionless

Megalin is a 600-kDa single transmembrane-domain receptor protein that belongs to the low-density lipoprotein receptor family. Megalin is responsible for the normal tubular reabsorption of filtered plasma proteins, thus preventing the loss of these essential molecules into the urine [108]. The almost complete clearance of proteins from the ultrafiltrate by megalin-driven endocytosis is accomplished in cooperation with the 460-kDa glycosylated protein receptor cubilin [109]. The normal function of cubilin is also dependent on the 38- to 50-kDa, single transmembrane protein amnionless that is essential for the trafficking of cubilin to the apical plasma membrane [110]. Megalin:cubilin:amnionless are expressed primarily in luminal plasma membranes of polarized absorptive epithelia [108 99]. Megalin binds a very wide range of ligands, including plasma transport proteins, peptides, hormones, etc. Known ligands of megalin normally filtered by the glomeruli include retinol-binding protein, transcobalamin-B12, insulin, α1- and β2-microglobulin, albumin, etc. (reviewed in [108]) (see Table 3). Although megalin and cubilin are structurally very different some of the ligands are shared with cubilin, whereas others are specific for either megalin or cubilin (reviewed in [108]). The receptors are co-expressed in several tissues, where they interact to function: Internalization of several cubilin ligands is strongly facilitated by megalin [108]. Megalin:cubilin:amnionless are highly expressed in the convoluted segments of the renal PT.

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Following binding to these receptors, ligands are internalized into coated vesicles and delivered to early and late endosomes. Whereas the receptors are recycled to the apical membrane, the ligands are transferred to late endosomes and lysosomes for protein degradation [108].

Reabsorption of filtered TBI in the renal PT has been mainly attributed to megalin-dependent cubilin-mediated endocytosis [16]. Yet, cubilin-independent megalin-mediated uptake of Tf may also occur [111]. Based on its plasma concentration and calculated glomerular sieving coefficient (GSC; derived from studies in patients with renal *Fanconi* syndrome) [17], the Tf concentration in the primary filtrate has been estimated to ~2 nM, which would allow its PT reabsorption via cubilin because this receptor binds Tf with a K_D of ~20 nM, as determined by surface plasmon resonance analysis [16]. The same applies to other filtered Fe-binding proteins (see above) that are known substrates of cubilin and/or megalin, including NGAL [112], albumin [111 113], and hepcidin [114]. Albumin requires cubilin for renal PT internalization, which is supported by experiments using cubilin-deficient mice [111]. The concentration of albumin in the glomerular filtrate has been calculated to ~53 nM [17] and the K_D of albumin to cubilin amounts to ~0.63 µM [115]. The plasma concentration of NGAL in healthy subjects amounts to ~6.5 µM [116] and should reach concentrations approximating ~0.65µM in the ultrafiltrate based on an estimated GSC of ~0.1 [17]. Surface plasmon resonance analysis has demonstrated binding of apo-NGAL to megalin with a K_D of ~60 nM [112], which is about 10-fold lower than the estimated NGAL concentration in the primary filtrate (see Table 3). Although, both lactoferrin [117]and hepcidin [114] bind to megalin and are likely to be filtered by the glomerulus (the latter completely), their binding affinity to megalin has not be determined. Overall, significant amounts of both TBI and NTBI are filtered by the glomerulus and likely to be reabsorbed via megalin:cubilin:amnionless in the PT (but see also section *7.1.2.* for Tf reabsorption).

TBI and NTBI that has been reabsorbed by the PT can meet four possible and not mutually exclusive fates (see [12] for a review): transcytosis; export back into the circulation via the Fe efflux transporter FPN1 aided by hephaestin (see section *7.1.5.*); storage in ferritin (see section *4.*); and utilization by PT cytosolic or mitochondrial Fe requiring processes (see section *7.1.3.*). *In vivo* transcytosis of Fe transporting proteins has been recently reported by a number of groups. Thus,

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albumin transcytosis in the PT was inferred from intravital tracking of fluorescent albumin by two-photon microscopy [118] as well as in a study showing the appearance of transgenic albumin specifically expressed in podocytes in the plasma where transcytosis was suggested to be mediated by a neonatal Fc (fragment crystallizable) receptor [119]. However, the issue of whether transcytosis of intact albumin actually occurs in renal PT is highly controversial [120, 121]. Whether bound Fe may be retained on albumin (and possibly other proteins) during such transcellular transfer, or may rather be released in some intracellular transit compartment, has, to our knowledge, not yet been investigated. Although transcytosis has also been reported for ferritin infused into the renal PT [122] this process is unlikely to play a role *in vivo* due to the large size and therefore poor glomerular filtration of ferritin (see section *6.*).

7.1.2. Transferrin receptor 1 (TfR1)

Renal PT cells express TfR1 at the apical membrane, at least in some species (and possibly in CD of kidney medulla) [103, 123], that could contribute to reabsorption of filtered TBI (see [12, 103] for reviews) considering the very high affinity of TfR1 to its natural ligand Tf (K_D ~0.2-0.4 nM) [124, 125]. A recent study with cultured PT cells, to date published only in abstract form [126], points to the possibility that, while megalin:cubilin mediated TBI reabsorption by the PT may predominate under Fe replete conditions, TfR1 becomes the principal receptor for Tf endocytosis under conditions of Fe restriction, due to differential regulation of these pathways by Fe [126].

7.1.3. DMT1 (SLC11A2)

The first mammalian Fe transporter protein DMT1 (divalent metal transporter 1) was identified by expression cloning [10]. DMT1 is a ferrous ion (Fe^{2+}) transporter that is energized by the H^+ electrochemical potential gradient while ferric ion (Fe^{3+}) is excluded [10]. However, H^+ coupling may not always be necessary for Fe^{2+} transport [127]. The role of DMT1 as a Fe^{2+} transporter was confirmed with Belgrade rats (*b*/*b*) and *mk* mice that carry a DMT1 G185R mutation, which results in deficient Fe^{2+} uptake and microcytic anaemia [68, 128]. DMT1 occurs in 4 major isoforms, which differ in their N- and C-termini. Isoforms 1A and 1B result from alternative 5' promoter usage with the translation of isoform

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1B actually starting at exon 2; alternative 3’ splicing yields two isoforms with different C-termini generated from transcripts containing (isoform I, +IRE) or lacking (isoform II, -IRE) an Fe-response element (IRE) in their 3’ untranslated region [129 130]. Functionally, the major human isoforms exhibit very similar characteristics when expressed in *Xenopus laevis* oocytes [131]. Although Gunshin *et al.* [10] demonstrated that, in addition to Fe^{2+}, a broad range of transition metals (including Cd^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Pb^{2+}) could evoke inward currents in *Xenopus* oocytes expressing rat DMT1, subsequent studies using a combination of voltage clamp, radiotracer and fluorescence assays in *Xenopus* oocytes or transfected HEK293 cells have established that human DMT1 is only capable of efficiently transporting Cd^{2+}, Fe^{2+} Co^{2+} and Mn^{2+} [132 11]. Human DMT1 exhibited the highest affinity for Cd^{2+} and Fe^{2+} ($K_{0.5}{}^{M}$ ≈1 µM) (see Table 2), showed moderately high affinity for Co^{2+} and Mn^{2+} ($K_{0.5}{}^{M}$ in the range 3–4 µM), whereas human DMT1 reacted with Ni^{2+}, VO^{2+}, and Zn^{2+} at lower affinity ($K_{0.5}{}^{M}$ in the range 10–20 µM). At −70 mV and at pH 5.5, the selectivity of human DMT1 metal-ion substrates were ranked $Cd^{2+} > Fe^{2+} > Co^{2+}$ and $Mn^{2+} \gg Zn^{2+}$, Ni^{2+}, VO^{2+} [11]. Whether DMT1 may also transport Cu^{2+} is still a matter of debate and may depend on the species and/or isoform investigated [11 133].

At the tissue level, DMT1 is ubiquitously expressed, most notably in the proximal duodenum, red blood cells, macrophages, but also in the kidneys and the brain [10]. DMT1 is expressed in the plasma membrane, typically in enterocytes, where it mediates Tf-independent Fe^{2+} absorption into the organism [10]. Alternatively, when DMT1 is located intracellularly, it is involved in the TfR1 pathway of Fe acquisition (as demonstrated in erythrocyte precursors or macrophages [67 134]) (see section *4*). There DMT1 is localized to intracellular endosomes and lysosomes that are formed during endocytosis of the Tf–TfR1 complex. Vacuolar-type ATPases acidify the endosomes and lysosomes which induces dissociation of Fe^{3+}. Fe^{3+} is reduced to Fe^{2+} by ferrireductase/oxidoreductase activity in the lumen of endosomes and lysosomes that is mediated by Steap proteins and that have also been found expressed at the mRNA level in epithelia, including the kidney [64 65]. This, in turn, activates DMT1 in the lysosomal membrane to co-transport the metal ion along with H^{+} into the cytosol [67 134].

The intracellular localization of DMT1 has also been demonstrated in epithelial cells [135], e.g. in immunolocalization studies of human 1B/+IRE and 1B/-IRE isoforms overexpressed in HEp-2 human

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larynx carcinoma cells [136]. In the kidney, the 1A/-IRE isoform was not detected by semiquantitative RT-PCR of total RNA from mouse kidney [130]. In contrast, we have detected all four DMT1 transcripts in RNA from rat renal cortex and a rat renal proximal tubule cell line, albeit with different abundance [137]. At the protein level, evidence could only be obtained for the presence of the +IRE isoforms in mouse kidney cortex and that were expressed at the apical pole of PT cells [138]. In another study, murine +IRE and -IRE DMT1 isoforms were transfected in LLC-PK1 cells: The +IRE isoform was associated with a higher surface expression and slower rate of internalization, as opposed to the -IRE isoform, which was efficiently sorted to recycling endosomes upon internalization, whereas the +IRE isoform was not efficiently recycled and rather targeted to lysosomes [139].

Consistent with a major role of megalin:cubilin dependent endocytosis for Tf clearance from the ultrafiltrate, DMT1 has been detected in late endosomes and lysosomes of rat kidney PT cells by electron microscopy and also mainly co-localized with late endosomal and lysosomal markers in a renal PT cell line [137]. Furthermore, a marked increase of punctate intracellular DMT1 immunostaining was observed in rat renal PT upon Fe deprivation, whereas DMT1 was decreased when animals were fed an Fe enriched diet [15]. Free Fe^{2+} has previously been postulated to be reabsorbed via DMT1 residing in the luminal membrane of mouse PT cells [138]. However, this localization contrasts with other reports indicating exclusive intracellular localization of DMT1, both in PT from rat [14 15 137] and mouse [140 12] (see Table 1 for an overview). Insufficient resolution of the immunohistochemical images in the study by Cannone-Hergaux and Gros [138] that could not distinguish between apical staining and staining of subapical vesicles has been proposed as a reason for this discrepancy [12]. Moreover, due to the high affinity of transferrin for Fe^{3+} [141], a brush-border membrane DMT1 could only reabsorb Fe from NTBI as Fe^{3+} that would also require its reduction by a brush-border ferrireductase and that has not been described in the kidney so far (with the exception of anecdotal evidence for a ferrireductase activity of a prion protein expressed in the apical membrane of PT cells [142]). Moreover, Wareing *et al.* [13] have performed tracer microinjections of $^{55}FeCl_3$ in the early PCT of rat kidney *in vivo* to determine the percentage of Fe reabsorption in the PT. Since urinary ^{55}Fe recovery was independent of the injection site (which varied between 1 and 6 mm from the glomerulus to the injection site) the

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authors concluded that Fe is not reabsorbed across the surface convolutions of the PT. This further argues against a role for apical DMT1 (and other Fe transporters) in non-protein bound NTBI reabsorption by the PT.

Mitochondria heavily rely on Fe-dependent metabolism and are therefore intracellular targets for Fe trafficking, which is particularly relevant in the kidney PT and thick ascending limb of LOH where mitochondria provide ATP for active reabsorption and secretion of solutes. Recently, we have obtained evidence for expression of the four major DMT1 isoforms in the OMM in several cell lines and tissues from multiple origin, including the kidney PT, and proposed that mitochondrial DMT1 represents a possible entry pathway for Fe and other metal ions utilized by mitochondria [85 86]. We used a variety of methods, including 1) cryo-immunogold electron microscopy to detect DMT1 co-localization with the OMM protein VDAC1; 2) confocal immunofluorescence microscopy to visualize partial co-localization of DMT1 with the mitochondrial markers VDAC1 and Tom6 (translocase of outer membrane 6); 3) immunoblotting of OMM and IMM fractions to demonstrate co-purification with the OMM marker VDAC1, but not with the IMM marker adenine nucleotide translocase; 4) a split ubiquitin yeast-two hybrid screen where the mitochondrial protein cytochrome C oxidase subunit II (COXII) was identified as an interaction partner of DMT1; 5) co-immunoprecipitation of COXII with DMT1 from cell lysates [85]. Most importantly, preliminary studies indicate that mitochondria isolated from stably DMT1-transfected HEK293 cells exhibit substantially higher uptake of the known DMT1 substrate $^{54}Mn^{2+}$ when the cells had been pretreated with doxycycline to induce the DMT1 promoter [143]. Moreover, $^{54}Mn^{2+}$ uptake into mitochondria from induced cells was sensitive to a specific DMT1 inhibitor [143]. Taken together, these data suggest that DMT1 not only exports Fe^{2+} (and Mn^{2+}) from endosomes and lysosomes, but also serves to import metal ions, including Mn^{2+} and Fe^{2+}, for mitochondrial utilization in the kidney PT and other tissues and cells.

Homozygous Belgrade rats (*b/b*) have a G185R mutation of DMT1 that diminishes transport and results in significantly increased serum Fe levels due to the inability of the tissues to utilize Fe [68 128]. These animals have been investigated to estimate the role of renal DMT1 in reabsorption of Fe, however with conflicting results. Belgrade rats showed significantly reduced renal kidney $^{59}Fe^{3+}$ 2

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hours after intravenous injection of Fe-Tf, compared to wild-type or heterozygous animals, suggesting that DMT1 is responsible for Fe uptake by renal tissue [144]. In contrast, another study showed urinary iron excretion rates that were unchanged in *b/b* compared to heterozygous animals [145]. This study may cast doubts on a functional role of DMT1 in reabsorption of Fe in the kidney, but alternate path(s) for Fe reabsorption by renal cells may also compensate for the lack of DMT1 protein. Indeed, significantly increased urinary Ca^{2+} excretion was measured in the Belgrade rats that did not show DMT1 dependence of urinary Fe excretion rates [145], which could be explained by increased competition of Fe^{2+} with Ca^{2+} for renal reabsorption by Ca^{2+} channels in the DT of DMT1-deficient Belgrade rats (see sections *7.3.1.* and *7.3.2.* for a further discussion). Another aspect needs also to be considered: A recent study with Belgrade rats has hinted to the fact that urinary Fe excretion increases with increasing age of the animals [146], suggesting a subtle but cumulative impact of DMT1 (dys)function on Fe handling by the kidney. This study may provide another explanation for the negative results described previously where young animals had been used [145]. Consequently, we investigated renal Fe handling in >25 weeks old Belgrade rats and their heterozygous litter mates and measured ~2-fold increased urinary Fe excretion (184 ± 40 versus 108 ± 9 µg/l x kg b.w.; n = 3) as well as ~2-fold decreased kidney Fe concentrations (0.39 ± 0.11 versus 0.21 ± 0.03 mg/g kidney tissue; n = 5) in Belgrade animals compared with heterozygous controls (F. Thévenod, A. R. Nair, W.-K. Lee & M.D. Garrick; *unpublished*), which is in agreement with those studies in Belgrade rats demonstrating the importance of DMT1 for renal Fe reabsorption [144 146].

7.1.4. ZIP8/ZIP14 (SLC39A8/SLC39A14)

Two other candidate transporters for non-protein bound Fe have been described in the apical membrane of renal PT, namely the Zrt, Irt-related proteins 8 (ZIP8/SLC39A8) and 14 (ZIP14/SLC39A14) [147]. Both carriers, are believed to operate as HCO_3^- coupled divalent metal ion cotransporters [148 149], and convincing experimental evidence has been provided that they are high-affinity Fe^{2+} transporters (for ZIP8 $K_{0.5}^{M}$ ≈ 0.7µM, for ZIP14 $K_{0.5}^{M}$ ≈2.3µM) when expressed in HEK-293H cells, Sf9 insect cells, or *Xenopus laevis* oocytes [150 151 152] (see Table 2). Yet, their subcellular

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localization in renal PT cells is not clear (see Table 1). Although Wang *et al.* [153] described the expression of ZIP8 in the BBM of PT cells by immunofluorescence staining of mouse renal cortical slices, the resolution of the images shown does not warrant this conclusion. Nor is it necessarily supported by the apical localization of the transporters in cell lines overexpressing ZIP8 and ZIP14. The *pros* and *cons* of plasma membrane ZIP8 and ZIP14 localization in cell lines and native tissues, such as kidney and liver, have been recently discussed [147]. Based on available evidence, the authors come to the conclusion that endogenous transporters may be more likely expressed in subapical endosomes and other intracellular organelles [147]. Clearly, more work is needed to define the subcellular localization of ZIP8 and ZIP14 in the PT.

7.1.5. Ferroportin (FPN1/SLC40A1)

Ferroportin1 (FPN1), also known as iron-regulated transporter 1 (IREG1) or metal transporter protein 1 (MTP1), was independently cloned by three groups in 2000 [38 39 40] (reviewed in [45]). To date, FPN1/SLC40A1 is the sole cellular Fe exporter described. Consistent with its assigned function, mammalian FPN1 was found expressed the basolateral pole of duodenal enterocytes and in splenic and hepatic macrophages by immunostaining [38 39]. Expression was also high in the basolateral membrane of the human placental syncytiotrophoblast, which is compatible with a role of FPN1 in Fe transfer to the fetal circulation [39]. Interestingly, despite the presence of a functional Fe-responsive element in its 5’ untranslated region [38 40 154], which allows translational repression of FPN1 by Fe-response proteins under conditions of low cytosolic Fe (see [155] for review), FPN1 was inversely regulated by Fe depletion in mouse duodenum and liver: Whereas Fe deprivation resulted in the expected downregulation of FPN1 in liver, duodenal FPN1 was strongly upregulated [38]. This apparent paradox was later resolved when a FPN1 transcript lacking the Fe-responsive element and specifically expressed in mouse duodenum was discovered that escapes repression by Fe depletion [156]. Additionally, FPN1 is regulated at the post-translational level by the hepatic hormone hepcidin [48] (see also section *3.*). Dietary Fe overload, inflammation and increased erythropoietic drive/anemia increase synthesis of hepcidin, which binds to FPN1 and leads to its internalization and subsequent

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lysosomal degradation, resulting in reduced dietary Fe absorption and Fe release from body Fe stores [48] (reviewed in [47]). FPN1-mediated Fe export into the circulation and subsequent binding to transferrin requires the presence of a ferrioxidase, namely hephaestin in duodenum [157] and ceruloplasmin in other cell types [158], that convert released Fe^{2+} to Fe^{3+} (reviewed in [159]). Although FPN1 has been cloned for ~15 years, relatively little is known about its functional characteristics: A human FPN1-enhanced green fluorescent protein fusion protein was expressed in *Xenopus* oocytes and was equally well permeated by microinjected $^{55}Fe^{2+}$ and $^{57}Co^{2+}$, and to some extent by $^{65}Zn^{2+}$ [41]. Notably, neither $^{109}Cd^{2+}$, $^{64}Cu^{2+}$ nor $^{54}Mn^{2+}$ were transported, even when applied at a concentration of 0.5mM [41] (see Table 2). FPN1-mediated efflux rate was found to be maximal at slightly alkaline pHo(utside) and abolished at pHo < 6.0, however, the mechanism of the pH effect on FPN1 transport is not understood [41].

Using thoroughly characterized affinity-purified rabbit polyclonal antibodies against rat FPN1, we have previously reported that FPN1 is expressed in rat PT (S2 > S1 >S3) where it is mainly localized in the basolateral plasma membrane (and some intracellular vesicles), as evidenced by immunohistochemistry and immunogold electron microscopy at high magnification [160]. Interestingly, FPN1 was absent from glomeruli and DT. Iron loading resulted in increased surface expression of FPN1 in a rat renal PT cell line, as detected by immunofluorescence labeling of non-permeabilized cells as well as surface biotinylation experiments, but with no change in total cellular FPN1 expression, suggesting that FPN1 redistributes to the cell surface and that increased insertion of FPN1 into the plasma membrane may play a role in protecting PT cells from Fe overload [160]. The basolateral localization of FPN1 in PT was subsequently confirmed in hepcidin$^{(-/-)}$ [123] and heme oxygenase 1$^{(-/-)}$ mice [161] using commercial antibodies, but FPN1 expression was much weaker in control animals. In contrast to those studies, Veuthey *et al.* showed both apical and basolateral FPN1 distribution in the mouse PT [140], and FPN1 was found only at the apex of PT in another mouse study [162]. In addition to the poor resolution of the images shown in these mouse studies, the specificity and quality of the antibodies used is difficult to assess as they were either from commercial sources and/or poorly characterized (Dr. B. Galy, European Molecular Biology Laboratory, Germany; *personal*

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communication) (information summarized in Table 1). There is also evidence to suggest that renal FPN1 expression is regulated by hepcidin: Intraperitoneal hepcidin pre-injection (24 h) prevents FPN1 upregulation induced by ischemia-reperfusion injury, as demonstrated in whole membranes of mouse kidney [163]. Furthermore, intraperitoneal hepcidin injection in mice induces a rapid (1 h) degradation of FPN1 in kidney homogenates (Drs. R.P.L. van Swelm & D.W. Swinkels, Department of Laboratory Medicine, RUMC, Nijmegen, The Netherlands, *personal communication; manuscript submitted*).

7.2. Iron transporters of the loop of Henle (LOH)

7.2.1. DMT1 (SLC11A2)

Very few studies have investigated the role of the LOH in Fe transport. Wareing et al. [13] performed two types of experiments: They used tracer microinjections of $^{55}FeCl_3$ in early PCT or early DCT of rat kidney *in vivo*, determined the percentage of urinary ^{55}Fe recovery (18.5 ± 2.9% for PCT versus 46.1 ± 6.1% for DCT) and by interpolation of the data calculated that the LOH contributes to ~40% of the total measured Fe transport. In addition, the authors microperfused LOHs *in vivo* with 7 μM $^{55}FeCl_3$ by placing the perfusion pipette at the last accessible convolution of the PCT and collected the perfusate at the first accessible portion of the DCT, the LOHs being isolated from the rest of the tubule by injection of mineral oil blocks into the tubule lumen [13]. By this approach they found that 52.7 ± 8.3% of perfused Fe was recovered from the DCT. This indicated that the LOH can reabsorb significant amounts of Fe. Furthermore, the same group found DMT1 expressed in the thick ascending limbs (TAL) of rat LOHs which exhibited punctate, DMT-1-specific immunoreactivity at the apical membrane and, more intensely, in the cytoplasm, and the intensity of staining increased progressively toward the DCT [14]. This suggests that apical DMT1 in the TAL cells of the LOH may reabsorb Fe. Interestingly, Fe overload in hepcidin$^{(-/-)}$ mice leads to increased Fe accumulation in TAL cells of the LOH as well as to increased basolateral FPN1 expression (see section *7.2.2.*) [123], suggesting that TAL cells of the LOH express a pathway for apical Fe uptake that may represent DMT1.

7.2.2. Ferroportin (FPN1/SLC40A1)

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The Fe efflux transporter FPN1 has been detected basolaterally and intracellularly in the thick ascending limb of the LOH in hepcidin$^{(-/-)}$ mice, but FPN1 expression was weak in the kidney cortex or medulla of control mice [123]. This study is in contrast to another study in mice in which no immunostaining of FPN1 was found in the LOH [140]. In our own studies in the rat kidney, we mainly focused on the FPN1 distribution in PT [160], but FPN1 was also expressed at low levels in the medulla, especially in the inner medulla (that includes the thin limbs of the LOH), as determined by immunoblotting (the possible reasons for the discrepancies in both mouse studies have been discussed in section *7.1.5.* and Table 1).

7.3. Iron transporters of the distal tubule (DT)

7.3.1. TRPV5 Ca^{2+} channels

TRPV5 (epithelial Ca^{2+} channel 1 ECaC1) belongs to the vanilloid (V) family of the transient receptor channel (TRP) superfamily. In humans, TRPV5 is considered the renal isoform of that family. The human TRPV5 (hTRPV5) gene encodes 729 amino acids, along with a predicted molecular mass of around 83 kDa. In the kidney, TRPV5 is localized at the apical membrane of DCT and connecting tubules where it contributes to active Ca^{2+} reabsorption [164] (see Table 1). TRPV5 is a highly Ca^{2+}-selective ($Permability_{Ca2+}$: $Permeability_{Na+}$ > 100), strongly inward rectifying cation channel with a single channel conductance between 55 and 107 pS [165, 166]. TRPV5 was shown to be permeable for Ca^{2+}, Ba^{2+}, $Sr^{2+}$and $Mn^{2+}$and inhibited by several di- and trivalent cations [165, 166]. The expression of functional TRPV5 is regulated by several hormones such as parathyroid hormone, and 1,25 dihydroxy vitamin D at the transcriptional level [164, 167] (reviewed in [168]). An orthologue to mammalian TRPV5 was cloned from the gill of pufferfish (*Fugu rubripes*) and characterized [169]. The *F. rubripes* ECaC (FrECaC) protein displays all structural features typical for mammalian ECaC. Functional expression of FrECaC in Madin-Darby canine kidney (MDCK) cells confirmed that the channel mediates Ca^{2+} influx, but FrECaC was also permeable to Fe^{2+} (and even better to Zn^{2+}). Bulk Fe flux was measured with ascorbic acid to prevent oxidation of $^{59}Fe^{2+}$ to $^{59}Fe^{3+}$, and a modest increase of Fe influx was observed when

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flux buffer contained 0.24 µM $^{59}Fe^{2+}$ (without Ca^{2+}) [169]. Thus FrECaC (and possibly renal TRPV5) may serve as a pathway for Fe acquisition.

7.3.2. Ca_v3.1 Ca^{2+} channels

Ca_v3.1 is a T (transient opening) -type Ca^{2+} channel, also known as α_{1G}. T-type channels differ from the L(long lasting) -type Ca^{2+} channels due to their ability to be activated by more negative membrane potentials, their small single channel conductance, and their unresponsiveness to Ca^{2+} antagonist drugs [170] [171]. As a member of the Ca_v3 subfamily of voltage-gated Ca^{2+} channels, T-type channels are important for the repetitive firing of action potentials in cells with rhythmic firing patterns such as cardiac muscle cells and neurons in the thalamus of the brain. Ca_v3.1 channels are widely expressed in excitable and non-excitable cells, including brain, ovary, placenta, heart, liver, bone, endocrine system and vascular smooth muscle [170] [172] [173]. Although Ca_v3.1 channels expression in the kidney has been primarily associated with the renal vasculature [174] one study revealed Ca_v3.1 expression in the DCT, in the connecting tubule and cortical collecting duct (CCD), and inner medullary collecting duct (IMCD) principal cells [175] (see Table 1). Using selective blockers, several reports have proposed that T-type Ca^{2+} channels are involved in steroid hormone-dependent luminal $^{45}Ca^{2+}$ uptake in isolated rabbit DCT [176] [177] [178].

Using a calcein-AM fluorescence assay to detect Fe in the cytosol under various Fe loading conditions, T-type calcium channels have been implicated in Fe^{2+} uptake by cardiomyocytes through the use of selective blockers [179]. In a more detailed study, Lopin *et al.* [180] examined the effects of extracellular Fe^{2+} on permeation and gating of Ca_v3.1 channels stably transfected in HEK293 cells, using whole-cell patch-clamp electrophysiology recording. In the absence of extracellular Ca^{2+}, Fe^{2+} carried detectable, whole-cell, inward currents at millimolar concentrations (73 ± 7 pA at -60 mV with 10 mM extracellular Fe^{2+}). With a two-site/three-barrier *Eyring* model for permeation of Ca_v3.1 channels [181], the authors estimated a transport rate for Fe^{2+} of ~20 ions/s for each open channel at -60 mV, with 1 µM extracellular Fe^{2+} and in the presence of physiological Ca^{2+} concentrations (2 mM extracellular Ca^{2+}). Reversal potentials indicated a Fe^{2+}/Ca^{2+} permeability ratio of 0.06-0.18. Because

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$Ca_v3.1$ channels exhibit a significant "window current" at resting membrane voltage (open probability, ~1%), the authors concluded that $Ca_v3.1$ channels represent a likely pathway for Fe^{2+} entry into cells at resting membrane potentials and possibly during the course of action potentials with clinically relevant concentrations of extracellular Fe^{2+} [180] (see Table 2).

7.3.3. DMT1 (SLC11A2)

The divalent metal transporter DMT1 (see sections *7.1.3.* and *7.2.1.*) has been found expressed in the luminal membrane of DCT. The most convincing localization study was performed by Ferguson *et al.* [14] in rat kidney. Using an affinity-purified rabbit polyclonal antibody directed against a 21-amino acid region in the NH_2 terminus of rat DMT-1 that should recognize all known DMT-1 isoforms [137], the authors showed extensive co-localization of DMT-1 and thiazide-sensitive Na^+-Cl^- cotransporter (NCCT) in the apical membrane of DCTs. DMT-1 was absent in late DCT to early connecting segments. Furthermore, in another study expression of DMT1 in the apical membrane and subapical region of rat DCT showed an inverse correlation with the dietary Fe content [15]. In support of a DCT localization of DMT1, *in vivo* tracer microinjection of ^{55}Fe into early rat DCT was associated with less than 50% urinary recovery, suggesting Fe reabsorption by late DCT segments and/or CD [13]. In contrast, two other studies have failed to confirm expression of DMT1 in DCT of the mouse [138, 140]. However, the immunohistochemical images used in those mouse studies showed poor resolution, and no attempt was made to identify the DMT1 labeled nephron segments with segment specific markers.

7.3.4. Lipocalin-2 receptor (SLC22A17)

The NGAL/24p3/lipocalin-2 receptor (Lip2-R) co-localizes with calbindin, a marker for late DCT and connecting tubule, in mouse and rat kidney [182]. The majority of DCT cells demonstrated co-localization of the two proteins, Lip2-R apically and calbindin intracellularly. Other cells however, were Lip2-R-positive but calbindin-negative, suggesting that Lip2-R is expressed in both early and late DCT. Since Lip2-R is predominantly expressed in CD of rodent kidney it will be discussed in that section (see section *7.4.2.*).

7.4. Iron transporters of the collecting duct (CD)

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7.4.1. DMT1 (SLC11A2)

Wareing *et al.* [13] attempted to identify the distal sites of renal Fe reabsorption by *in vivo* tracer microinjection of $^{55}FeCl_3$ into the DCT segment of the rat nephron *in vivo*. Approximately 50% of the ^{55}Fe injected was recovered in the urine, suggesting that Fe is significantly reabsorbed by nephron segments distal to the DCT. This functional mapping of the distal nephron sites of Fe reabsorption is in good agreement with the immunofluorescence distribution of DMT1 in nephron segments in subsequent studies by the same group. Ferguson *et al.* [14] [145] and Wareing *et al.* [15] demonstrated strong DMT-1-specific immunofluorescence in the cortical and outer medullary CD. The signal gradually decreased in intensity from the cortex to the outer stripe and inner stripe of the medulla. The distribution of DMT1 in different cell types of the CD varied considerably: Co-localization with the water channel aquaporin 2 showed that DMT-1 is present apically and intracellularly in principal cells of CD in the cortex and outer medulla. In superficial cortex, DMT-1 also co-localized with the vacuolar-type H^+-ATPase at the apical membrane, thus indicating expression in A-intercalated cells, and also showed a bipolar distribution in some, but not all, B-intercalated cells. In the outer medullary region, DMT-1 was less intense at the apical membrane and more diffuse throughout the cytosol in intercalated cells. DMT-1 immunoreactivity decreased progressively along the length of the CD, and inner medullary CD ducts showed only faint DMT-1-specific staining [14]. Another study confirmed DMT-1 in the renal medulla in mice [140], but no information was provided on the cell types associated with DMT-1 in renal CD. The apical expression of DMT1 in type A intercalated cells of the cortical CD is interesting from a physiological point of view considering that Fe^{2+} transport by DMT1 is coupled to H^+ [10]. Type A-intercalated cells could provide the pH gradient necessary to drive DMT1-mediated luminal Fe^{2+} uptake. Furthermore, the localization of DMT1 in type A-intercalated cells would be compatible with the recently postulated function of these CD cells as a critical barrier against infection [183] by depleting Fe from the urine that is necessary for bacterial growth. Fe^{2+} clearance from the lumen mediated by H^+-driven Fe^{2+} uptake via DMT1 would represent another defense mechanism in addition to the suggested secretion of the Fe-bacterial siderophore

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sequestering peptide lipocalin-2/24p3/NGAL (neutrophil gelatinase-associated lipocalin) and urinary acidification as processes responsible for bacteriostasis [183] (see section *7.4.2.*).

7.4.2. Lipocalin-2 receptor (SLC22A17/Lip2-R)

Neutrophil gelatinase-associated lipocalin (NGAL [human]/siderocalin/24p3 [rodent]/lipocalin-2 [human]) (Lip2) was discovered in neutrophils [105] and was also shown to be induced in intestinal epithelia by inflammation or cancer [184] . Lip2 binds Fe^{3+} through association with bacterial [185] and mammalian siderophores [73 186], thereby affecting Fe homeostasis of target cells and their survival and proliferation. Hence, Lip2 may play a role as an Fe-sequestering protein in antibacterial innate immunity by decreasing susceptibility to bacterial infections [185 187 188], and its interactions with bacterial siderophores have been very well characterized [189]. Lip2 may also deliver Fe to epithelia of the primordial kidney [190], stimulate growth and differentiation, and promote repair and regeneration of damaged epithelia [191]. Therefore, Lip2 is increasingly used as a sensitive biomarker of kidney damage in clinical settings [192 193]. During renal insults, e.g. acute kidney injury (AKI), Lip2 is thought to be secreted by the distal nephron (DCT and CD) and excreted into the urine [194] although earlier studies from the same laboratory had emphasized that Lip2 is secreted by the PT during AKI [195 191]. It has been postulated that Lip2 is secreted, possibly to limit injury and promote Fe-dependent regeneration of damaged epithelia [191], but how this happens is unclear. Despite a wealth of publications, the function of Lip2 in the kidney in health and disease as well as its mechanisms of secretion are still not well understood.

The mounting relevance of Lip2 in the medical field has increased the interest in identifying putative receptors of this ligand. Megalin, the epithelial multi-ligand receptor expressed in renal PT (see *7.1.1.*) binds Lip2 with high affinity [112]. In addition, a receptor for murine Lip2, Lip2-R, has also been cloned [196] whose mRNA encodes 520 amino acids (molecular mass ~60 kDa and 11 or 12 transmembrane domains depending on the predicted topology) and whose affinity for Lip2 is ~1000x higher (K_D ~90pM) [197] than that of megalin (K_D ~60nM) [112]. According to the SLC (solute carrier) nomenclature system this receptor is also named SLC22A17 or BOCT (brain organic cation

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transporter) [198]. However, classical substrates of organic cation transporters are not transported by SLC22A17 ([199] and N.A. Wolff & F. Thévenod; *unpublished*). Interestingly, several short N- and C-terminal splicing variants (22 kDa and ~30 kDa, respectively) of the Lip2-R have been described in humans and rodents, respectively [196] [200], but their function in health or disease is unknown. Although Lip2-R protein is expressed in the kidney [196] its localization and functions in that organ were unknown until recently. Using two affinity-purified polyclonal rabbit antibodies directed against the N- and C-terminal domains of Lip2-R, we showed apical expression of Lip2-R in rodent kidney DCT (where it co-localized with calbindin, Lip2-R being expressed apically and calbindin intracellularly) and CD (mainly inner medullary CD), but not in PT (where it was found weakly expressed intracellularly). In DCT, some cells were Lip2-R-positive but calbindin-negative, suggesting that Lip2-R is expressed in both early and late DCT (see Table 1). Lip2-R was also found expressed in respective mouse cell lines (mDCT209; mIMCD3, mCCDcl1), but not in PT cell lines (WKPT-0293 Cl.2) ([182] and *unpublished*). We also confirmed the expression of several immunoreactive protein bands in purified plasma membranes by immunoblotting (MM ~35 kDa, ~45 kDa, ~60 kDa and ~130 kDa), thus confirming the presence of “short” and “long” forms of the protein that may represent splicing variants or dimers of the receptor, respectively ([182] and *unpublished*). Chinese hamster ovary (CHO) overexpressing Lip2-R or mDCT209 cells expressing Lip2-R endogenously internalized submicromolar concentrations of fluorescence-labelled Tf, albumin, or MT and their uptake was blocked by 500 pM Lip2 [182], which confirms that the uptake of these proteins is mediated by the Lip2-R. Using microscale thermophoresis, a powerful technique to quantify biomolecular interactions [201], we showed that MT binds to Lip2-R with a K_D of ~100nM [182]. Hence, Lip2-R seems to bind proteins filtered by the kidney, including Tf and MT, with high affinity and may contribute to receptor-mediated endocytosis of these proteins as well as of Lip2 in the distal nephron (see Table 2).

Is the uptake of metalloproteins, such as Tf, Lip2 or MT, by Lip2-R physiologically and pathophysiologically relevant when bulk protein reabsorption is thought to take place in the PT? Experimental evidence has demonstrated that physiologically a small but significant proportion of filtered proteins is reabsorbed by the distal segments of the nephron [202] [203] [204] [205]. Although

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megalin:cubilin:amnionless is a high-capacity receptor complex for endocytotic reabsorption of filtered proteins [206] some proteins/metalloproteins may bypass reabsorption in the PT, either as the consequence of their low affinity to megalin and low concentration in the ultrafiltrate (e.g. MT with a K_D of ~5-100 µM [207] but a plasma concentration of ~ 0.5-5 nM [208 209]) (in this context see Table 3) or due to limited reabsorptive capacity of the system (e.g. following glomerular or PT damage and ensuing proteinuria) [210 211 17]. A high-affinity protein receptor in the distal nephron such as Lip2-R could contribute to exhaustive protein/metalloprotein reabsorption and deplete the final urine from protein-bound Fe (and other metals) under physiological conditions, or limit losses associated with renal diseases, including various forms of inherited or acquired *Fanconi* syndrome [17]. Indeed, two *in vivo* studies have demonstrated Fe uptake into the distal nephron of nephrotic rats [212] or following glomerular damage induced by acute Fe overload [213]. Interestingly, Fe deposits were found in lysosomes of DT by electron microscopy [212] and kidney medullary tubule cells by histochemistry [213]. Furthermore, in hepcidin$^{(-/-)}$ mice, a model of the Fe overload disease hemochromatosis, Fe deposits were also found in the distal nephron [123]. Hence, increased uptake of proteins/metalloproteins by Lip2-R in the distal nephron could initiate or enhance kidney injury. Along these lines, a recent *in vivo* study has implicated the Lip2-R in the CD in contributing to initiation and/or aggravation of renal inflammation and fibrosis in response to proteinuria [214].

Correnti *et al.* [74] have recently questioned a role of Lip2 in cellular Fe metabolism based on their observation that gentisic acid (a putative mammalian siderophore) could not form a stable ternary complex with Lip2 and Fe and on their inability to demonstrate any physical interaction between Lip2 and N- (NTD) or C-terminal domains (CTD) of mouse Lip2-R by surface plasmon resonance analyses. However, using the 105 residue NTD of human Lip2-R and analysis of its interaction by microscale thermophoresis, isothermal titration calorimetry and nuclear magnetic resonance, we could demonstrate binding of human Lip2 to its cellular receptor NTD (A.-I. Cabedo Martinez *et al.*; *submitted*). Although the affinity we measured between human Lip2-R-NTD and human Lip2, i.e. ~7 µM for apo-Lip2 and ~20 µM for holo-Lip2 (Lip2 bound to the bacterial siderophore enterobactin) suggests that the N-terminus alone cannot account for the internalization

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of Lip2 by Lip2-R and that other parts of the receptor must contribute to the interaction our results are in contradiction with the conclusions of Correnti *et al.* [74]. We suspect that their failure to observe any direct interaction between Lip2 and mouse Lip2-R results from 1) their inability to control the state of their recombinant Lip2 (apo- or holo-) and 2) a lack of proper formation of the disulfide bridges of their mouse Lip2-R-NTD preparation, as the formation of aberrant disulfides would probably lead to forms of mouse Lip2-R-NTD that are unable to bind to Lip2 (A.-I. Cabedo Martinez *et al.*; *submitted*). Either or both of these points could explain their inability to observe an interaction between Lip2 and mouse Lip2-R-NTD. Overall, our data suggest that Lip2-R represents a high-affinity multiligand receptor for apical endocytosis of proteins and/or metalloproteins (such as Tf or Cd^{2+}-MT) in renal epithelia. Increased endocytosis subsequent to glomerular and/or PT damage may promote renal epithelial damage by death, inflammation and fibrosis.

7.4.3. Ferroportin (FPN1/SLC40A1)

Strong FPN1 (see sections *7.1.5.* and *7.2.2.*) immunostaining has been detected in inner medullary CD of mice but FPN1 expression decreased in anemic mice [140]. Specific immunostaining was found intracellularly. Outer medulla showed intracellular staining as well. In contrast, no medullary FPN1 staining was detected in another study in mice [123] and FPN1 expression was weak in inner medulla of rat kidney when measured by immunoblotting in our own studies [160]. The discrepancies observed in these mouse studies have been discussed in section *7.1.5.* and Table 1.

8. Cadmium toxicity

8.1. General considerations and link to iron transport

Pollution by cadmium (Cd) is rising worldwide because of intensified industrial activities that have increased its availability and because Cd cannot be degraded further [7 215]. Chronic exposure to low Cd concentrations is a significant health hazard for ~10% of the world population that increases morbidity and mortality [216]. Indeed, Cd damages multiple organs in humans and other mammalian organisms by causing nephrotoxicity, osteoporosis, neurotoxicity, genotoxicity, teratogenicity, or endocrine and reproductive defects [217].

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In mammalian organisms, Cd is a toxic-only element with no known role in physiological processes: As a non-essential metal ion Cd^{2+} competes with essential metal ions in cells where it disrupts cellular functions and leads to disease. Although Cd^{2+} is not capable of catalyzing *Fenton* chemistry in biological systems, it may initiate free radical chain reactions by depleting endogenous redox scavengers, inhibiting anti-oxidative enzymes, blocking the mitochondrial electron transport chain, and/or displacing redox active metals, such as Fe^{2+} or Cu^{2+} from their carrier proteins [218] and thereby trigger cell death by apoptosis (reviewed in [219]). Cd^{2+} can also substitute for Ca^{2+} in cellular signaling or for Zn^{2+} in many enzymes and transcription factors which may account for some of the biological effects of Cd^{2+} [219 220]. In order for toxicity to occur Cd^{2+} must first enter cells by utilizing transport pathways for essential metals, such as Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} or Mn^{2+}, that are present in biological systems mostly as complexes with small organic molecules or as metalloproteins. These metal ion compounds are hydrophilic and must permeate lipophilic cellular membranes through intrinsic proteinous pathways. Hence, free or small complexed metal ions may be transported via ion channels or carrier proteins whereas metalloproteins are taken up by receptor-mediated endocytosis (RME). Cd^{2+} has similar physico-chemical properties as essential metal ions (for a detailed account see [18] and references therein) and Cd^{2+} complexes are analogous to endogenous biological molecules, therefore this attribute has been termed "ionic and molecular mimicry" [4 221]. Hence, transport (and toxicity) of Cd^{2+} can only occur if cells possess pertinent transport pathways for essential metals or biological molecules. A number of pathways has been suggested to allow Cd^{2+} entry in excitable and non-excitable cells [9] and the most likely candidates have been recently reviewed [18, 215].

Chronic exposure to Cd^{2+} involves very low concentrations of Cd^{2+} that originate from environmental pollution and mainly results from dietary sources and cigarette smoking. Hence Cd^{2+} enters the body primarily through the lungs and the gastrointestinal (GI) tract: The absorption of Cd^{2+} from the lungs is much more effective than that from the gut; however, Cd^{2+} absorption from the GI tract is the main route of Cd^{2+} exposure in humans [215]. Following absorption in the lungs and/or intestine, Cd^{2+} in the blood at first largely binds to albumin and other thiol-containing HMWP and low

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molecular weight proteins (LMWP) in the plasma, including MT, as well as to blood cells. But Cd^{2+} tends to concentrate in blood cells (mainly erythrocytes) and <10% remains in the plasma [222]. Since intravenously injected MT-bound Cd^{2+} in mice is quickly cleared from the plasma by the kidneys [223] this protein fraction in the circulation – that is assumed to originate from Cd^{2+} stored in liver cells as Cd^{2+}-MT and is released from damaged cells (see below) – has been thought to be of great importance for the transport of Cd^{2+} to the kidney during long-term exposure [224] [225] [226] (although the plasma Cd^{2+}-MT concentrations following injections exceeded physiological MT concentrations by >2000-fold [209] [208]; see section *8.2.* for a critical discussion). The blood level of Cd^{2+} largely reflects recent Cd^{2+} exposure with a half-life of 75-128 days [227]. It ranges between 0.03 and 0.5 µg/l (~0.3-5 nM) depending on the preparation method and the populations studied (reviewed in [228]) and its concentration in plasma will be at least ten-fold lower [222].

Cd^{2+} reaching the plasma is thought to be initially transported to the liver where intracellular Cd^{2+} induces the synthesis of the endogenous detoxicant MT, which binds, sequesters and detoxifies Cd^{2+} because its affinity to Cd^{2+} is very high with a K_D of ~10^{-14} M (reviewed in [229]). Yet, a small proportion of liver (Cd^{2+}-)MT is assumed to be slowly released into blood plasma as the hepatocytes in which Cd^{2+} is sequestered die off, either through normal turnover or as a result of Cd^{2+} injury [224, 230, 231]. Several studies have demonstrated that following long-term exposure to Cd^{2+} and even at long time intervals after a single exposure, the level of Cd^{2+} is initially highest in the liver and then gradually increases in the kidneys [232] [233]. The strongest evidence for the concept that the major source of renal Cd^{2+} during chronic Cd^{2+} exposure is derived from hepatic Cd^{2+}, which is transported in the form of Cd^{2+}-MT in blood plasma, was derived from studies with transplanted livers of Cd^{2+}-exposed rats to normal rats [234]. Cd^{2+} and MT in the liver of recipient rats decreased over time after surgery whereas renal Cd^{2+} and MT levels increased and most of the Cd^{2+} in the kidney was bound to MT [234]. Although none of these data proved that redistribution of Cd^{2+} from the liver to the kidney is mediated by circulating Cd^{2+}-MT, this hypothesis still prevails in the literature (see section *8.2.* for a critical discussion).

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Once absorbed Cd^{2+} is stored in various organs, including the kidneys and liver, with a half-life of several decades [2 7 215]. This happens because Cd^{2+} induces the expression of detoxifying molecules that form a complex with the metal ion and thereby alleviate its toxic effects. But this apparently beneficial effect is a two edged-sword because these seemingly harmless Cd^{2+} complexes represent an endogenous source of high concentrations of potentially toxic Cd^{2+}. The major detoxifying tool of the cell for Cd^{2+} complexation is MT. MTs are low-molecular weight (MM ranging from 3.5-14 kDa), cysteine-rich metal-binding proteins that have the capacity to bind both physiological Zn^{2+} ions and toxic Cd^{2+} ions through the thiol group of its cysteine residues that represent nearly 30% of its amino acidic residues [100 235 236].

8.2. Cadmium handling by the kidney

As a consequence of its storage in tissues Cd^{2+} is very poorly excreted, mainly in urine and feces. With low, or even moderate, levels of exposure, little or no Cd^{2+} is excreted in the urine [237], which indicates that Cd^{2+} is reabsorbed and stored by the kidney. In humans, the amount of Cd^{2+} excreted daily in urine represents only about 0.005-0.015% of the total body burden [237] and amounts to 0.05-0.2 µg/l (reviewed in [228]). Most of the Cd^{2+} in urine is bound to MT [238 239] and it is assumed that urinary Cd^{2+} and MT stem from filtered Cd^{2+}-MT and normal turnover and shedding of epithelial cells, or - perhaps - from exosomes derived from Cd^{2+}-MT containing tubule epithelia. This supposition is based on chronic studies in several mammalian species showing that urinary excretion of Cd^{2+} increases slowly for a considerable time as a reflection of the level of Cd^{2+} exposure and the body burden of the toxic metal ion, which correlates with an increase of Cd^{2+} in the renal cortex (reviewed in [240]). But when the concentration of Cd^{2+} in the renal epithelial cells reaches a threshold value of ~150-200 µg/g wet weight Cd^{2+} disrupts tubular reabsorptive processes and the excretion of Cd^{2+} and MT begin to increase in a linear manner, which is associated with the onset of polyuria and proteinuria [241 242] (reviewed in [240]). When kidney dysfunction aggravates and a sharp increase in excretion of Cd^{2+} and MT occurs a decrease in renal and liver Cd^{2+} concentrations is also observed [243]

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[244]. Hence, the early, slow linear phases of Cd^{2+} and MT excretion likely mirror the level of chronic Cd^{2+} exposure whereas the later sharp increases in excretion reflect Cd^{2+}-induced tubular injury.

In the previous paragraphs it has been emphasized that chronic exposure to low environmental or dietary Cd^{2+} concentrations results in accumulation of the metal ion in the kidney with a biological half-life of ~20 years or more [2 7 215] where it may cause damage, fibrosis or failure [245 246], or – with Cd^{2+} being a Class 1 human carcinogen - cancer [247]. In contrast, acute or subchronic Cd^{2+} nephrotoxicity is associated with a general transport defect of the PT that mimics the *de Toni-Debré-Fanconi*-Syndrome [248 249] with proteinuria, aminoaciduria, glucosuria and phosphaturia (for review, see [250]).

For several decades the following scenario has prevailed to account for acute or chronic Cd^{2+} toxicity in the kidney: It has been presumed that Cd^{2+} in the circulation is filtered by the glomerulus because of the small molecular mass of most circulating Cd^{2+} forms: In the plasma, Cd^{2+} is thought to be loosely associated with molecules, such as LMWP - e.g. β-2 microglobulin, α-1 microglobulin, retinol-binding protein, insulin or parathyroid hormone - with amino acids or the sulfhydryl compounds GSH or cysteine, or tightly bound to specific metal-binding proteins such as the LMWP MT [229]. Several HMWP, e.g. albumin, bind Cd^{2+} with low affinity [251], also show some degree of glomerular filtration [17] and may therefore carry Cd^{2+} into the ultrafiltrate. Furthermore, the Fe-binding protein Tf that is filtered by the glomerulus (see section *6.*) may also bind Cd^{2+} in plasma [252 253 254]. The PT largely contributes to the reabsorption of Cd^{2+} because as the first segment of the nephron it is responsible for bulk reabsorption of primary urine, which mainly takes place by solvent drag via paracellular routes (see section *5.*). But PT cells may also possess apical transporters (as proposed for ZIP8 and ZIP14 transporters that carry both Fe^{2+} and Cd^{2+} [148 149]; however see section *7.1.4.* and [147] for a note of caution), amino acid transporters, metabolizing brush-border enzymes (such as γ-glutamyl transpeptidase that degrades GSH), and the receptor for protein endocytosis megalin:cubilin:amnionless [108] that mediate apical uptake of Cd^{2+} ions and Cd^{2+} complexes (see section *7.1.1.*, and [9 18] for reviews). There is also evidence that Cd^{2+} is taken up at the basolateral surface of PT cells [255 256] and it has recently been shown to take place via the organic cation

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transporter 2 (OCT2) [257 258]. Although it is mechanistically remarkable that an organic cation transporter is able to carry a divalent metal ion as a substrate, a K_m value of ~54 µM for Cd^{2+} [258] suggests that the *in vivo* toxicological relevance of this transporter is questionable.

Like other LMWP, Cd^{2+}-MT/MT is thought to be reabsorbed from primary urine into PT cells of the kidneys by megalin:cubilin:amnionless receptor-mediated endocytosis [259 206 260 261] (see Table 3). Studies with cultured PT cells have provided evidence that Cd^{2+}-MT/MT is trafficked to acidic late endosomes and lysosomes [262 263] where MT may be degraded by lysosomal proteases whereas Cd^{2+} may exit the endosomal/lysosomal compartment by DMT1-mediated efflux into the cytosol [137 264]. This may cause acute PT toxicity in cases where PT cells would have to handle high concentrations of endocytosed Cd^{2+}-MT [264]. However, if the Cd^{2+}-MT stress is low PT cells may adapt by inducing the upregulation of detoxifying proteins, including MT [265] that inactivate and complex Cd^{2+} released from lysosomes into the cytosol for long-term storage [266]. Cd^{2+} accumulation in the PT (and storage as Cd^{2+}-MT) may be likely further promoted by the absence of an efflux pathway for cytosolic Cd^{2+} into the extracellular fluid or blood plasma because FPN1 that is expressed at the basolateral cell side of PT cells [160] does not transport Cd^{2+} (as opposed to Fe^{2+}) [41] (see also section *7.1.5.*).

The concept that endocytosis of filtered Cd^{2+}-MT by megalin:cubilin:amnionless is mainly responsible for accumulation of Cd^{2+} in the PT was based on studies demonstrating redistribution of hepatic Cd^{2+} to the kidney that was supposed to be Cd^{2+}-MT [232 233 234], on *in vivo* animal studies with intravenously injected Cd^{2+}-MT [223 267 268 269 270 271 272] as well as on microinjections of Cd^{2+}-MT in isolated PT [273]. It was confirmed and elaborated in cell culture studies [260 261 262 263] (reviewed in [274]). However, all of the *in vivo* and cell culture studies applied Cd^{2+}-MT at micromolar concentrations. Meanwhile surface plasmon resonance analyses, cell culture and *in vivo* studies have established that the binding affinity of megalin for MT is ~100 µM [207 260 272], which is compatible with the observations from the *in vivo* animal studies. But considering that plasma concentrations of MT are in the range of ~ 0.5-5 nM [208 209] in humans (and healthy laboratory animals), the concept that filtered (Cd^{2+})-MT is also taken up by PT via megalin:cubilin:amnionless-dependent endocytosis under physiological conditions [275] is unfounded, and thus the current models of Cd^{2+} accumulation (and chronic toxicity)

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in the PT should be revised (nevertheless Cd^{2+}-MT may still be useful as a model compound to study RME of Cd^{2+}-protein complexes in cell culture or *in vivo* as the high binding affinity of MT to Cd^{2+} precludes dissociation of toxic free Cd^{2+}). It is more likely that other LMWP (e.g. α1- or β2-microglobulin) and albumin, which also bind Cd^{2+} and reach submicromolar concentrations in plasma and ultrafiltrate [276 277], are more relevant ligands (e.g. β2-microglobulin binds to megalin with a K_D of ~0.42 µM [278]) (see also Table 3) that are endocytosed by megalin:cubilin:amnionless to induce Cd^{2+} accumulation and eventually PT toxicity. It could be argued that these proteins exhibit relatively low affinities to Cd^{2+} compared to MT (reliable K_D values of ~10^{-6} M for Cd^{2+} and other divalent metal ions are only available for albumin and β2-microglobulin [251 279]), indicating that at steady-state maximally 1% of these proteins in the circulation will form complexes with blood Cd^{2+} (with a concentration of 0.3-5 nM [228]) whereas MT in the circulation will be Cd^{2+}-saturated (based on equivalent low nM concentrations of MT and Cd^{2+} and a K_D of ~10^{-14} M [229]). Yet the relatively high concentration of microglobulins and albumin in the primary filtrate and their high binding affinity to megalin (see Table 3) combined with the multiplicative effect of their continuous glomerular filtration makes them more prone to accumulate in the PT and contribute to chronic renal PT toxicity than Cd^{2+}-MT whose concentration in the primary filtrate is at least 10^5-times lower [208 209] than its K_D for megalin binding [207].

Because only 0.02-03% of filtered proteins, including MT (based on measured values for plasma and urinary MT 99.7% of filtered MT must be reabsorbed by the kidney [208 209]), are excreted with the urine [280] (reviewed in [281]) additional uptake pathways for proteins and protein-Cd^{2+} complexes must exist in the distal nephron (see below).

Cd^{2+} may not be only toxic to PT cells, but also to glomeruli and the distal nephron [282]. Glomerular damage with a decreased GFR has been observed in occupationally exposed workers [283] and in environmentally exposed populations where it may occur at similar Cd^{2+} dose levels as the tubular damage [284 246]. But overall, the pathogenesis of the glomerular lesion in Cd^{2+} nephropathy is not well understood [285]. Downstream segments of the nephron, both in the cortex and medulla, also exhibit a high permeability to Fe^{2+} and other metal ions (see sections *7.2.-7.4.*) and could hence

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contribute to uptake of Cd^{2+}. This would be particularly the case when proximal segments of the nephron are defective or "overwhelmed" as a consequence of increased filtration (e.g. due to glomerular damage). Under those circumstances, later segments should become more relevant for uptake. As an example, a chronic *in vivo* study in ducks demonstrated substantial damage to glomerular podocytes following exposure to a combination of lead, methylmercury and cadmium that was associated with enhancement of degenerative changes in PT and CD; in contrast exposure to cadmium alone showed no podocyte damage and tubular damage was restricted to PT whereas the CD was not affected [286]. Sporadic evidence for chronic Cd^{2+} toxicity of the distal portions of the nephron induced by Cd^{2+} exposure has also been obtained, both in experimental animals [287 288] and in Cd^{2+}-exposed workers [289], but the mechanisms of distal nephron damage remain unclear.

In agreement with studies demonstrating *in vivo* ^{55}Fe transport in the rat LOH [13] that may be mediated by apical DMT1 [14] (see section *7.2.1.*), Barbier *et al.* [290] performed $^{109}Cd^{2+}$ tracer microinjections into the late PCT and early DCT of rat kidney and $^{109}Cd^{2+}$ reabsorption in the LOH was obtained by calculating the difference between $^{109}Cd^{2+}$ recovery after early DCT and late PCT. The authors obtained 46.8% unidirectional $^{109}Cd^{2+}$ fluxes that were reduced to 25.4% in the presence of 100 µM Fe^{2+}, suggesting that DMT1 in the LOH is involved in $^{109}Cd^{2+}$ uptake (and competes with Fe^{2+} for reabsorption) [290]. This report is unique for its exhaustive characterization of Cd^{2+} transport by the nephron, but unfortunately no additional studies have been published to confirm its conclusions. Despite variable and partly questionable results of FPN1 expression and localization in the rodent LOH [140 160 123] (see section *7.2.2.*), even if FPN1 were expressed in the LOH Cd^{2+} would remain trapped within the cells of the LOH because it is not transported by FPN1 [41] (see section *7.1.5.*).

Given the large number of Ca^{2+} channels expressed throughout the body, the importance of Ca^{2+} signaling, and the large number of ions a channel can transport (~10^5 ions/s), even slight permeability of a Ca^{2+} channel to Cd^{2+} might lead to significant Cd^{2+} entry. Indeed, several Ca^{2+} channels that are expressed in the apical membrane of DCT [164 175] are known to transport Cd^{2+}. T-type Ca^{2+} channels are blocked by Cd^{2+} [291 292], but their role in Cd^{2+} transport had not been investigated until recently. $Ca_v3.1$, also known as α_{1G}, is a T type Ca^{2+} channel and is expressed in the DT (see *7.3.2.*). $Ca_v3.1$ channels

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may be suitable for Cd^{2+} transport, because they have a well-defined and substantial window current at negative membrane potentials at which the driving force for divalent cation entry is high [293] and they are ~2-fold less selective for Ca^{2+} than are L-type Ca^{2+} channels [170], which suggests that Cd^{2+} may have an increased chance of permeating the channel in the presence of competing Ca^{2+}. Furthermore, development of resistance to Cd^{2+} in cell culture has been linked to down-regulation of $Ca_v3.1$, which suggested the involvement of this channel in Cd^{2+} toxicity [294]. Consequently, Lopin *et al.* [295] examined the effects of extracellular Cd^{2+} on permeation and gating of $Ca_v3.1$ channels stably transfected in HEK293 cells, by using whole-cell recording. In the absence of other permeant ions (Ca^{2+} and Na^{+} were replaced by N-methyl-D-glucamine), Cd^{2+} carried sizable inward currents through Cav3.1 channels (210±20 pA at -60 mV with 2 mM Cd^{2+}). Incubation with radiolabeled $^{109}Cd^{2+}$ confirmed uptake of Cd^{2+} into cells with $Ca_v3.1$ channels. With a two-site/three-barrier Eyring model for permeation of $Ca_v3.1$ channels [181], a transport rate for Cd^{2+} of ~1 ion/s was estimated for each open channel at -60 mV, with 3-10 nM extracellular Cd^{2+} and in the presence of 2 mM extracellular Ca^{2+}. On the basis of the Goldman-Hodgkin-Katz theory [296], a Cd^{2+}/Ca^{2+} permeability ratio of 0.66 was calculated, with Cd^{2+} being only slightly less permeable than Ca^{2+} [295]. Blood Cd^{2+} concentrations range between 0.3 and 5 nM (reviewed in [228]). Following glomerular filtration, the concentration of the "free" ionic form of Cd^{2+} in the primary urine of the nephron may increase up to 15-fold in the lumen of the DT (see below). In addition, luminal ionic Cd^{2+} may be further increased by its release from small peptides that are degraded by brush-border enzymes (such as γ-glutamyl transpeptidase that degrades GSH). Hence, in view of the significant "window current" at negative voltages and the high permeability of $Ca_v3.1$ channels for Cd^{2+} at low nanomolar concentrations (in the presence of physiological Ca^{2+} concentrations), these channels are a likely candidate pathway for Cd^{2+} entry into cells expressing $Ca_v3.1$ channels, including the kidney DT. Thus, $Ca_v3.1$ channels could significantly contribute to the *in vivo* renal toxicity of Cd^{2+} (see Table 2). In another study, the human TRPV5 (ECaC1) of the vanilloid family of the transient receptor channel (TRP) superfamily was transiently expressed in the plasma membrane of human embryonic kidney (HEK293) cells [297] (see also section *7.3.1.*). Cd^{2+} (and less well Zn^{2+}) permeated hTRPV5 in ion imaging experiments using Fura-2 or

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Newport Green DCF (dichlorofluorescein) with an EC_{50} of ~10 respective ~100 µM Cd^{2+} depending on the absence or presence of 1mM Ca^{2+} in the extracellular medium. The results were further confirmed using whole-cell patch clamp technique. Transient overexpression of hTRPV5 sensitized cells to Cd^{2+} toxicity. Hence, although micromolar concentrations of Cd^{2+} appear to be required for permeation the results suggest that TRPV5 may also play a role in Cd^{2+} uptake by the DCT, especially under low Ca^{2+} dietary conditions, when these channels are maximally upregulated. Functional studies *in vivo* support these cell culture studies. Using both, $^{109}Cd^{2+}$ and $^{45}Ca^{2+}$ tracer microinjections into the early and late DCT of rat kidney and recovery in the urine, Barbier *et al.* showed about 20-25% unidirectional reabsorption of either of the tracers in the DCT that were almost completely abolished in the presence of 100 µM Fe^{2+} or 20 µM Cd^{2+}, respectively (which, of course, seems too high from a viewpoint of physiological relevance) [290]. This suggests that Cd^{2+} is taken up by Fe^{2+} and/or Ca^{2+} transporters in the DCT, possibly DMT1 that is expressed in the luminal membrane of this nephron segment [14] [15] (see section *7.3.3.*), but also TRPV5/Ca_v3.1 Ca^{2+} channels (see above). However, the experimental design of this tracer microinjection study could not exclude that DMT1 expressed in the CD may also mediate $^{109}Cd^{2+}$ reabsorption [290] (see below). Finally, we have previously shown apical expression of the Lip2-R in rodent kidney DT (see sections *7.3.4.* and *7.4.2.*) and cultured mDCT209 cells expressing Lip2-R at their surface internalized submicromolar concentrations of fluorescence-labelled MT that was blocked by 500 pM of the endogenous ligand Lip2 [182]. And Cd^{2+}-MT caused cell death in mDCT209 cells that could be rescued by 500 pM Lip2 [182]. Hence, it is possible that Lip2-R contributes to receptor-mediated endocytosis of toxic Cd^{2+}-MT and other Cd^{2+}-protein complexes in the DT.

Cd^{2+} reabsorption by terminal nephron segments, i.e. CD, has been investigated by Barbier *et al.* [290] using $^{109}Cd^{2+}$ and $^{45}Ca^{2+}$ tracer microinjections. Unidirectional $^{45}Ca^{2+}$ fluxes in the terminal segments of the nephron were not affected by 20 µM Cd^{2+}, which suggests that Cd^{2+} permeating Ca^{2+} channels are less likely expressed in the CD. In contrast, they showed that 50-100 µM Fe^{2+}, Co^{2+} and Zn^{2+} increased ^{109}Cd recovery in the urine after microinjection in the early DCT. This suggests involvement of DMT1 in nephron segments downstream of the early DCT, i.e. late DCT and CD (see

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section *7.4.1.*). Mouse IMCD3 cells are sensitive to $CdCl_2$ (LC_{50} ~ 40 µM), indicating uptake of Cd^{2+} by these CD cells [298] although the toxic concentrations of Cd^{2+} in these cells were much higher than the K_m of DMT1 for Cd^{2+} transport of ~ 1 µM [132]. Although Lip2-R is expressed in CD (see section *7.4.2.*) and mediates uptake and toxicity of Cd^{2+}-MT in various cultured cells [182, 299], its role in Cd^{2+}-MT transport and cell damage in the CD has not been investigated so far. The likely Fe transport pathways of the distal nephron segments (LOH, DT, CD) that compete with Cd^{2+} for uptake are summarized in Table 2.

A difficulty in the attempt to estimate the role of distal nephron segments (LOH, DT, CD) in Cd^{2+} uptake - and given the binding affinity of putative transport pathways for Cd^{2+} - is the inability to determine accurately the actual concentrations of ionic and complexed forms of Cd^{2+} in nephron segments downstream of the PT. Nevertheless, an approximation can be obtained from the ratio of inulin concentration in the tubule fluid over plasma (TF/P). Inulin is an indicator of the GFR, i.e. a molecule that is only filtered by the glomerulus and neither reabsorbed nor secreted by the nephron. The TF/P ratio of inulin therefore reflects fluid reabsorption by the nephron [300]. The TF/P ratio of inulin increases from 1 to 3 at about 2/3 of the PT length. It reaches a value of 7 at the beginning of the DT and increases up to ~15 towards its end to reach a final value of 10-200 in the final urine depending on the diuresis condition (water- and anti-diuresis, respectively). In other words, the concentration of non-reabsorbed solutes increases by a factor of 3 along the PT, varies between 7 and 15 along the DT and can increase up to 200-fold in the CD. Consequently, the concentrations of Cd^{2+} and MT/Cd^{2+}-MT may increase up to 10-15-fold in the DT and up to 200-fold in the CD, suggesting that these nephron segments may be more relevant segments of the kidney cortex for Cd^{2+} and MT/Cd^{2+}-MT uptake and accumulation under conditions of chronic low Cd^{2+} exposure than previously thought. DMT1 and the Lip2-R expressed in DT (cortex) and CD segments (cortex and medulla) are more likely to efficiently reabsorb Cd^{2+} and Cd^{2+}-MT because of their high affinity to these Cd^{2+} compounds (K_m of DMT1 for Cd^{2+} ~ 1 µM [132] [11]; K_D of Lip2-R for MT ~ 120 nM [182]). But the renal medulla also accumulates significant amounts of both Cd^{2+} and (Cd^{2+}-)MT in humans and concentrations of both Cd^{2+} compounds can reach ~50% of the levels found in the cortex [301] [302].

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Indeed, MT has been detected by immunohistochemistry in distal segments of the nephron using cortical and medullary sections of rodent and human kidney [303 304 305] (although no co-localization with nephron segment-specific markers was performed) and whose expression was increased by exposure to Cd^{2+} [304 305]. But why then is Cd^{2+} nephrotoxicity less apparent in the DT and kidney medulla? The relative resistance of the DT and kidney medulla to Cd^{2+} toxicity may result from their lower sensitivity to oxidative stress [306 307], their increased potential for adaptive responses and stress-induced factors (e.g. hypoxia-inducible factor-1α (HIF-1α), hepcidin, neutrophil gelatinase-associated lipocalin (NGAL) to name a few) [21 308 309], and their metabolic profile (largely anaerobic glycolysis due to a low partial pressure for O_2 in the medullary segments) [310]. All these issues are known to account for the resistance of the DT and kidney medulla to acute tubular damage (e.g. of mitochondria) [311] and necrosis (outer medulla (straight segment of PT, medullary thick ascending limb of LOH) > cortex (PT, DT) >> inner medulla) elicited by various inducers of AKI (reviewed in [312]).

9. Determinants of the fate of the kidney exposed to iron or cadmium

Irrespective of the nature of the Cd^{2+} compound that is taken up by tubule cells, its impact on cell viability differs from the effect of Fe. Both metal ions appear to be taken up as protein-metal complexes via RME. Moreover, both metal ions may accumulate intracellularly and be detoxified by binding to high-affinity chaperone proteins: For instance, the intracellular Fe storage protein ferritin is induced by overload of the PT with Fe *in vivo* [313 314 54 123]. Similarly, following Cd^{2+} exposure *in vivo* PT cells upregulate the scavenger protein MT for Cd^{2+} storage [234 235]. Both proteins even share some degree of overlapping specificity for Cd^{2+} and Fe^{2+}: Apart from binding Fe with high affinity [91] ferritin also binds Cd^{2+} [253 315 254]. Conversely, although MT binds Cd^{2+} with very high affinity [316] it has the ability to form complexes with Fe^{2+} as well [317]. Yet, it is surprising that acute or chronic Fe overload generally does not cause manifest renal damage [213 314] whereas nephrotoxicity is not an unusual sequel of acute or chronic Cd^{2+} exposure [245 246 250]. Transport (TfR1, megalin:cubilin:amnionless, Lip2-R, DMT1, $Ca_v3.1$, etc.) (compare sections *7.* and *8.* and Table 2) and detoxification/storage mechanisms (MT, ferritin) are shared by Fe^{2+} and Cd^{2+}. Hence, the differential toxicity of Fe^{2+} and Cd^{2+}

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in PT cells could be attributed to disparate expression and/or upregulation of the intracellular metal scavenger proteins ferritin and MT [318 319 320], which are both regulated by antioxidant response elements in their gene promoter region (reviewed in [91 235]). In addition, the differences in binding characteristics of these chaperone proteins for Fe and Cd^{2+} could be accountable: Both, the binding capacity (maximally ~4500 for ferritin [91] versus 7 metal ion binding sites for MT [321]) and/or in the binding affinities of ferritin or MT to Fe and Cd^{2+} [322 317] are at variance. However, currently there is no stringent evidence for the relative contribution of both chaperone proteins in determining the extent of Fe and Cd^{2+} toxicity in PT cells, therefore further work is needed to clarify these issues.

In contrast, obvious differences concern the cellular utilization or non-utilization (i.e. toxicity) of both metal ions, and the efflux pathway for Fe^{2+} and Cd^{2+}. Fe^{2+} enters mitochondria possibly via DMT1 in the OMM [85] and mitoferrins in the IMM [87] for synthesis of heme and Fe–sulfur clusters [79 80]. In contrast, after crossing the OMM (possibly via DMT1 in the OMM [85]) and entering the mitochondrial matrix through the mitochondrial Ca^{2+} uniporter in the IMM [323] Cd^{2+} disrupts mitochondrial function [324 325], which leads to increased formation of ROS and death through apoptosis and/or necrosis (reviewed in [326 219]). Another principle difference is the inability of FPN1 at the basolateral side of PT cells to transport Cd^{2+} into the circulation and thereby to clear it from the cell, which is in contrast to FPN1 handling of Fe^{2+} [41]. Hence, these differences may underlie - or at least contribute to - the mode of damage likely developing after acute exposure to high concentrations of Cd^{2+} (necrosis) or to nephrotoxicity induced by chronic accumulation of low concentrations of Cd^{2+} (apoptosis, cancer development).

Considering the competition between Fe^{2+} and Cd^{2+} for transport at renal entry pathways ("ionic and molecular mimicry")[4 221], it should also be deduced that Fe deficiency may not only augment body and kidney Cd^{2+} burden [5] by increased gastrointestinal absorption of Cd^{2+} [327] but also facilitate renal Cd^{2+} reabsorption and thereby elicit a higher likelihood of renal tubule damage.

10. Outlook

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Despite a wealth of novel data suggesting a contribution of the kidney to systemic Fe homeostasis and good evidence for uptake and reabsorption of Fe by specific transporters in various nephron segments as well as their involvement in Cd^{2+} uptake and nephrotoxicity, there is still not enough *in vivo* data available. Presently, a detailed characterization of Fe transporters has been performed in cell lines and heterologous expression systems and these studies have unambiguously demonstrated which transporters are Fe^{2+} and Cd^{2+} selective and which are not (see Table2). In contrast, only one study has described *in vivo* transport of Fe by the nephron and this report is already 15 years old [13]. Similarly, only one study has investigated the role of different nephron segments in uptake of Cd^{2+} and other divalent metal ions *in vivo* [290]. These studies – as exhaustive and thorough as they are - would need to be confirmed and extended, in particular by using nephron specific transporter knockout models. Indeed, this approach has been successfully used to clarify the role of megalin:cubilin:amnionless in reabsorption of protein by the PT [278] [115] [328], including the Fe-binding protein Tf [16]. Unfortunately, no study has attempted to investigate systemic Fe homeostasis in nephron specific megalin or megalin:cubilin deficient animals or whether the PT of these animals is protected against Cd^{2+} nephrotoxicity. Studies are underway that aim to investigate the role of renal Lip2-R in the uptake and toxicity of metalloproteins, including Cd^{2+}-MT and Tf, in nephron specific lip2-R knockout mice, (F. Thévenod & S. de Seigneux; *in preparation*).

Another weakness of this area of research is the inconsistent characterization of the renal localization of renal Fe transporters that has resulted in contradictory results (see Table 1). These conflicting data have even inspired some authors to indiscriminately adopt models of renal Fe transport that are not compatible with renal epithelial physiology and membrane transport. A critical analysis of the relevant experimental reports identifies four principle methodological problems that may also be linked: The tissue sections used for immunostaining were often of poor quality (e.g. tubule lumina were collapsed); authors failed to properly identify nephron segments, e.g. by co-localization studies with nephron specific markers; the images showed poor resolution; and last not least, antibodies used were often of doubtful origin or their specificity had not been proven (see Table 1). This lack of methodological rigor (obviously immunostaining data were not important

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enough and therefore did not seem to concern the authors) has, at least in part, weakened this field of research. Once more, the use of renal Fe transporter knockout models will hopefully shed light on these confusing data.

Nevertheless, this review has clearly demonstrated that the kidney plays a previously unsuspected role in systemic iron balance and that renal Fe transporters are crucial for the accumulation of Cd^{2+} in the kidney and the development of nephrotoxicity. Future studies, as suggested above, should be able to verify the significance of Fe transporters described in this review and possibly identify additional relevant uptake pathways for renal Fe transport. Last not least, we think that the contribution of circulating Cd^{2+}-MT (originating from the liver or not) to chronic Cd^{2+} accumulation in the PT and its toxicity may not be as important as previously suggested and that other hypotheses should be envisaged and experimentally tested.

11. Acknowledgments

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12. Abbreviations

2,5-DHBA	2,5-dihydroxybenzoic acid
AKI	acute kidney injury
AQP2	aquaporin 2
ATP	adenosine triphosphate
BBM	brush-border membrane
BOCT	brain organic cation transporter (Lip2-R/Lipocalin-2 receptor)
calcein-AM	calcein acetoxymethyl (AM) ester

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$Ca_V3.1$	calcium channel, voltage-dependent (T-type, α_{1G} subunit)
CCD	cortical collecting duct
CD	collecting duct
CHO	chinese hamster ovary (cell line)
COXII	cytochrome C oxidase subunit II
CTD	carboxy-terminal domain
DCF	dichlorofluorescein
DCT	distal convoluted tubule
DMT1/Nramp2/DCT1/SLC11A2	proton-coupled divalent metal transporter 1
DT	distal tubule
EC_{50}	half maximal effective concentration
ECaC	epithelial calcium channel
ENaC	epithelial sodium channel
Fc	fragment crystallizable (region of an antibody)
FLVCR1	feline leukemia virus, subgroup C, receptor (heme exporter)
FPN1/IREG1/MTP1/SLC40A1	ferroportin
GFR	glomerular filtration rate
GI	gastrointestinal
Grx3_4	glutaredoxin 3_4
GSC	glomerular sieving coefficient
GSH	glutathione
H-_L-ferritin	heavy-_light ferritin subunit
HEK	human embryonic kidney (cell line)
HIF-1α	hypoxia-inducible factor-1α
HMWP	high-molecular weight protein
IMM	inner mitochondrial membrane
IRE	iron response element

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$K_{0.5}^{M}/K_m$	substrate/metal concentration at which velocity is half-maximal
K_D	equilibrium dissociation constant
LC_{50}	half maximal lethal concentration
Lip2	lipocalin-2 (NGAL/24p3)
Lip2-R	lipocalin-2 receptor
LMWP	low-molecular weight proteins
LOH	loop of Henle
MDCK	Madin-Darby canine kidney (cell line)
MFRN1/SLC25A37	mitoferrin-1
MM	molecular mass
MT	metallothionein
NCCT	NaCl cotransporter
NGAL/24p3	neutrophil gelatinase-associated lipocalin
NTBI	non-transferrin-bound iron
NTD	amino-terminal domain
OCT2	organic cation transporter 2
OMCD	outer medullary collecting duct
OMM	outer mitochondrial membrane
pA	picoampere
PCBP1	poly (rC)-binding protein 1
PCT	proximal convoluted tubule
pS	picosiemens
PT	proximal tubule
RME	receptor-mediated endocytosis
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
S1_2_3	segment 1_2_3 (PT)

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SLC	solute carrier
Steap	sixtransmembrane epithelial antigen of the prostate (oxidoreductase)
T_L-type	transient opening_long lasting -type (calcium channel)
TAL	thick ascending limb
TBI	transferrin-bound iron
Tf	transferrin
TF/P	tubule fluid over plasma
TfR1_2	transferrin receptor 1_2
TOM6	translocase of outer membrane 6
TRPML1/ML1/MLN1/MCLN1	transient receptor potential mucolipin 1
TRPV	vanilloid (V) family of the transient receptor potential channel (TRP) superfamily
VDAC	voltage-dependent anion channel/porin
ZIP8_14/SLC39A8_14	Zrt, Irt-related proteins 8 (ZIP8/SLC39A8) and 14

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13. References

1. T. Ganz, *Physiol Rev*, 2013, **93**, 1721-1741.
2. G. F. Nordberg, *Toxicol Appl Pharmacol*, 2009, **238**, 192-200.
3. I. Sovago and K. Varnagy, *Met Ions Life Sci*, 2013, **11**, 275-302.
4. T. W. Clarkson, *Annu Rev Pharmacol Toxicol*, 1993, **33**, 545-571.
5. L. Jarup, M. Berglund, C. G. Elinder, G. Nordberg and M. Vahter, *Scand J Work Environ Health*, 1998, **24 Suppl 1**, 1-51.
6. M. Berglund, A. Akesson, B. Nermell and M. Vahter, *Environ Health Perspect*, 1994, **102**, 1058-1066.
7. L. Jarup and A. Akesson, *Toxicol Appl Pharmacol*, 2009, **238**, 201-208.
8. G. L. Diamond, in *Molecular biology and toxicology of metals*, eds. R. K. Zalups and J. Koropatnick, Taylor & Francis, London and New York, 2000, ch. 11, pp. 300-345.
9. R. K. Zalups and S. Ahmad, *Toxicol Appl Pharmacol*, 2003, **186**, 163-188.
10. H. Gunshin, B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan and M. A. Hediger, *Nature*, 1997, **388**, 482-488.
11. A. C. Illing, A. Shawki, C. L. Cunningham and B. Mackenzie, *J Biol Chem*, 2012, **287**, 30485-30496.
12. C. P. Smith and F. Thévenod, *Biochim Biophys Acta*, 2009, **1790**, 724-730.
13. M. Wareing, C. J. Ferguson, R. Green, D. Riccardi and C. P. Smith, *J Physiol*, 2000, **524 Pt 2**, 581-586.
14. C. J. Ferguson, M. Wareing, D. T. Ward, R. Green, C. P. Smith and D. Riccardi, *Am J Physiol Renal Physiol*, 2001, **280**, F803-F814.
15. M. Wareing, C. J. Ferguson, M. Delannoy, A. G. Cox, R. F. McMahon, R. Green, D. Riccardi and C. P. Smith, *Am J Physiol Renal Physiol*, 2003, **285**, F1050-F1059.
16. R. Kozyraki, J. Fyfe, P. J. Verroust, C. Jacobsen, A. Dautry-Varsat, J. Gburek, T. E. Willnow, E. I. Christensen and S. K. Moestrup, *Proc Natl Acad Sci U S A*, 2001, **98**, 12491-12496.

Metallomics Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

17. A. G. Norden, M. Lapsley, P. J. Lee, C. D. Pusey, S. J. Scheinman, F. W. Tam, R. V. Thakker, R. J. Unwin and O. Wrong, *Kidney Int*, 2001, **60**, 1885-1892.

18. F. Thévenod, *Biometals*, 2010, **23**, 857-875.

19. J. Wang and K. Pantopoulos, *Biochem J*, 2011, **434**, 365-381.

20. K. Gkouvatsos, G. Papanikolaou and K. Pantopoulos, *Biochim Biophys Acta*, 2012, **1820**, 188-202.

21. A. M. Martines, R. Masereeuw, H. Tjalsma, J. G. Hoenderop, J. F. Wetzels and D. W. Swinkels, *Nat Rev Nephrol*, 2013, **9**, 385-398.

22. I. De Domenico, D. McVey Ward and J. Kaplan, *Nat Rev Mol Cell Biol*, 2008, **9**, 72-81.

23. K. Pantopoulos, S. K. Porwal, A. Tartakoff and L. Devireddy, *Biochemistry*, 2012, **51**, 5705-5724.

24. in *Iron in biochemistry and medicine, II.*, eds. A. Jacobs and M. Worwood, Academic Press, London, 1980, pp. 1-706.

25. J. Prousek, *Pure Appl Chem.*, 2007, **79**, 2325–2338.

26. R. Green, R. Charlton, H. Seftel, T. Bothwell, F. Mayet, B. Adams, C. Finch and M. Layrisse, *Am J Med*, 1968, **45**, 336-353.

27. M. Hynes, *J Clin Pathol*, 1948, **1**, 57-67.

28. M. C. Linder, *Nutrients*, 2013, **5**, 4022-4050.

29. C. Finch, *Blood*, 1994, **84**, 1697-1702.

30. M. Shayeghi, G. O. Latunde-Dada, J. S. Oakhill, A. H. Laftah, K. Takeuchi, N. Halliday, Y. Khan, A. Warley, F. E. McCann, R. C. Hider, D. M. Frazer, G. J. Anderson, C. D. Vulpe, R. J. Simpson and A. T. McKie, *Cell*, 2005, **122**, 789-801.

31. A. Qiu, M. Jansen, A. Sakaris, S. H. Min, S. Chattopadhyay, E. Tsai, C. Sandoval, R. Zhao, M. H. Akabas and I. D. Goldman, *Cell*, 2006, **127**, 917-928.

32. A. Rajagopal, A. U. Rao, J. Amigo, M. Tian, S. K. Upadhyay, C. Hall, S. Uhm, M. K. Mathew, M. D. Fleming, B. H. Paw, M. Krause and I. Hamza, *Nature*, 2008, **453**, 1127-1131.

Metallomics Accepted Manuscript

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60

33. A. Shawki, P. B. Knight, B. D. Maliken, E. J. Niespodzany and B. Mackenzie, *Curr Top Membr*, 2012, **70**, 169-214.

34. D. J. Lane, D. H. Bae, A. M. Merlot, S. Sahni and D. R. Richardson, *Nutrients*, 2015, **7**, 2274-2296.

35. B. Mackenzie, A. Shawki, R. Kim, S. R. Anthony, P. B. Knight, E. M. Bradford and G. E. Shull, *Faseb J*, 2011, **25**, 238.231 (Abstract).

36. L. Vanoaica, D. Darshan, L. Richman, K. Schumann and L. C. Kuhn, *Cell Metab*, 2010, **12**, 273-282.

37. H. Shi, K. Z. Bencze, T. L. Stemmler and C. C. Philpott, *Science*, 2008, **320**, 1207-1210.

38. S. Abboud and D. J. Haile, *J Biol Chem*, 2000, **275**, 19906-19912.

39. A. Donovan, A. Brownlie, Y. Zhou, J. Shepard, S. J. Pratt, J. Moynihan, B. H. Paw, A. Drejer, B. Barut, A. Zapata, T. C. Law, C. Brugnara, S. E. Lux, G. S. Pinkus, J. L. Pinkus, P. D. Kingsley, J. Palis, M. D. Fleming, N. C. Andrews and L. I. Zon, *Nature*, 2000, **403**, 776-781.

40. A. T. McKie, P. Marciani, A. Rolfs, K. Brennan, K. Wehr, D. Barrow, S. Miret, A. Bomford, T. J. Peters, F. Farzaneh, M. A. Hediger, M. W. Hentze and R. J. Simpson, *Mol Cell*, 2000, **5**, 299-309.

41. C. J. Mitchell, A. Shawki, T. Ganz, E. Nemeth and B. Mackenzie, *Am J Physiol Cell Physiol*, 2014, **306**, C450-459.

42. Z. L. Harris, A. P. Durley, T. K. Man and J. D. Gitlin, *Proc Natl Acad Sci U S A*, 1999, **96**, 10812-10817.

43. C. D. Vulpe, Y. M. Kuo, T. L. Murphy, L. Cowley, C. Askwith, N. Libina, J. Gitschier and G. J. Anderson, *Nat Genet*, 1999, **21**, 195-199.

44. S. Cherukuri, R. Potla, J. Sarkar, S. Nurko, Z. L. Harris and P. L. Fox, *Cell Metab*, 2005, **2**, 309-319.

45. N. Montalbetti, A. Simonin, G. Kovacs and M. A. Hediger, *Mol Aspects Med*, 2013, **34**, 270-287.

Metallomics Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

46. E. Ramos, L. Kautz, R. Rodriguez, M. Hansen, V. Gabayan, Y. Ginzburg, M. P. Roth, E. Nemeth and T. Ganz, *Hepatology*, 2011, **53**, 1333-1341.

47. T. Ganz and E. Nemeth, *Biochim Biophys Acta*, 2012, **1823**, 1434-1443.

48. E. Nemeth, M. S. Tuttle, J. Powelson, M. B. Vaughn, A. Donovan, D. M. Ward, T. Ganz and J. Kaplan, *Science*, 2004, **306**, 2090-2093.

49. P. Brissot, M. Ropert, C. Le Lan and O. Loreal, *Biochim Biophys Acta*, 2012, **1820**, 403-410.

50. B. J. Scott and A. R. Bradwell, *Clin Chem*, 1983, **29**, 629-633.

51. C. Hershko and T. E. Peto, *Br J Haematol*, 1987, **66**, 149-151.

52. R. C. Hider, *Eur J Clin Invest*, 2002, **32 Suppl 1**, 50-54.

53. C. A. Finch, V. Bellotti, S. Stray, D. A. Lipschitz, J. D. Cook, M. J. Pippard and H. A. Huebers, *West J Med*, 1986, **145**, 657-663.

54. L. A. Cohen, L. Gutierrez, A. Weiss, Y. Leichtmann-Bardoogo, D. L. Zhang, D. R. Crooks, R. Sougrat, A. Morgenstern, B. Galy, M. W. Hentze, F. J. Lazaro, T. A. Rouault and E. G. Meyron-Holtz, *Blood*, 2010, **116**, 1574-1584.

55. B. S. Skikne, *Am J Hematol*, 2008, **83**, 872-875.

56. D. M. Frazer and G. J. Anderson, *Biofactors*, 2014, **40**, 206-214.

57. A. N. Luck and A. B. Mason, *Curr Top Membr*, 2012, **69**, 3-35.

58. P. Aisen, *Int J Biochem Cell Biol*, 2004, **36**, 2137-2143.

59. J. E. Levy, O. Jin, Y. Fujiwara, F. Kuo and N. C. Andrews, *Nat Genet*, 1999, **21**, 396-399.

60. H. Kawabata, R. Yang, T. Hirama, P. T. Vuong, S. Kawano, A. F. Gombart and H. P. Koeffler, *J Biol Chem*, 1999, **274**, 20826-20832.

61. J. Chen and C. A. Enns, *Biochim Biophys Acta*, 2012, **1820**, 256-263.

62. B. D. Grant and J. G. Donaldson, *Nat Rev Mol Cell Biol*, 2009, **10**, 597-608.

63. P. Ponka, C. Beaumont and D. R. Richardson, *Semin Hematol*, 1998, **35**, 35-54.

64. R. S. Ohgami, D. R. Campagna, E. L. Greer, B. Antiochos, A. McDonald, J. Chen, J. J. Sharp, Y. Fujiwara, J. E. Barker and M. D. Fleming, *Nat Genet*, 2005, **37**, 1264-1269.

65. R. S. Ohgami, D. R. Campagna, A. McDonald and M. D. Fleming, *Blood*, 2006, **108**, 1388-1394.

Metallomics Accepted Manuscript

66. S. Dhungana, C. H. Taboy, O. Zak, M. Larvie, A. L. Crumbliss and P. Aisen, *Biochemistry*, 2004, **43**, 205-209.

67. S. Gruenheid, F. Canonne-Hergaux, S. Gauthier, D. J. Hackam, S. Grinstein and P. Gros, *J Exp Med*, 1999, **189**, 831-841.

68. M. D. Fleming, M. A. Romano, M. A. Su, L. M. Garrick, M. D. Garrick and N. C. Andrews, *Proc Natl Acad Sci U S A*, 1998, **95**, 1148-1153.

69. X. P. Dong, X. Cheng, E. Mills, M. Delling, F. Wang, T. Kurz and H. Xu, *Nature*, 2008, **455**, 992-996.

70. W. Breuer, M. Shvartsman and Z. I. Cabantchik, *Int J Biochem Cell Biol*, 2008, **40**, 350-354.

71. F. Petrat, H. de Groot, R. Sustmann and U. Rauen, *Biol Chem*, 2002, **383**, 489-502.

72. R. C. Hider and X. L. Kong, *Biometals*, 2011, **24**, 1179-1187.

73. L. R. Devireddy, D. O. Hart, D. H. Goetz and M. R. Green, *Cell*, 2010, **141**, 1006-1017.

74. C. Correnti, V. Richardson, A. K. Sia, A. D. Bandaranayake, M. Ruiz, Y. Suryo Rahmanto, Z. Kovacevic, M. C. Clifton, M. A. Holmes, B. K. Kaiser, J. Barasch, K. N. Raymond, D. R. Richardson and R. K. Strong, *PLoS One*, 2012, **7**, e43696.

75. U. Muhlenhoff, S. Molik, J. R. Godoy, M. A. Uzarska, N. Richter, A. Seubert, Y. Zhang, J. Stubbe, F. Pierrel, E. Herrero, C. H. Lillig and R. Lill, *Cell Metab*, 2010, **12**, 373-385.

76. D. R. Richardson, D. J. Lane, E. M. Becker, M. L. Huang, M. Whitnall, Y. Suryo Rahmanto, A. D. Sheftel and P. Ponka, *Proc Natl Acad Sci U S A*, 2010, **107**, 10775-10782.

77. A. S. Zhang, A. D. Sheftel and P. Ponka, *Blood*, 2005, **105**, 368-375.

78. A. D. Sheftel, A. S. Zhang, C. Brown, O. S. Shirihai and P. Ponka, *Blood*, 2007, **110**, 125-132.

79. R. S. Ajioka, J. D. Phillips and J. P. Kushner, *Biochim Biophys Acta*, 2006, **1763**, 723-736.

80. R. Lill, B. Hoffmann, S. Molik, A. J. Pierik, N. Rietzschel, O. Stehling, M. A. Uzarska, H. Webert, C. Wilbrecht and U. Muhlenhoff, *Biochim Biophys Acta*, 2012, **1823**, 1491-1508.

81. W. Xu, T. Barrientos and N. C. Andrews, *Cell Metab*, 2013, **17**, 319-328.

82. R. Benz, *Biochim Biophys Acta*, 1994, **1197**, 167-196.

83. W. Tan and M. Colombini, *Biochim Biophys Acta*, 2007, **1768**, 2510-2515.

Metallomics Accepted Manuscript

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52
53
54
55
56
57
58
59
60

84. M. Y. Liu and M. Colombini, *J Bioenerg Biomembr*, 1992, **24**, 41-46.

85. N. A. Wolff, A. J. Ghio, L. M. Garrick, M. D. Garrick, L. Zhao, R. A. Fenton and F. Thévenod, *Faseb J*, 2014, **28**, 2134-2145.

86. N. A. Wolff, L. M. Garrick, L. Zhao, M. D. Garrick and F. Thévenod, *Channels (Austin)*, 2014, **8**, 458-466.

87. G. C. Shaw, J. J. Cope, L. Li, K. Corson, C. Hersey, G. E. Ackermann, B. Gwynn, A. J. Lambert, R. A. Wingert, D. Traver, N. S. Trede, B. A. Barut, Y. Zhou, E. Minet, A. Donovan, A. Brownlie, R. Balzan, M. J. Weiss, L. L. Peters, J. Kaplan, L. I. Zon and B. H. Paw, *Nature*, 2006, **440**, 96-100.

88. P. N. Paradkar, K. B. Zumbrennen, B. H. Paw, D. M. Ward and J. Kaplan, *Mol Cell Biol*, 2009, **29**, 1007-1016.

89. J. Sripetchwandee, S. B. KenKnight, J. Sanit, S. Chattipakorn and N. Chattipakorn, *Acta Physiol (Oxf)*, 2014, **210**, 330-341.

90. S. B. Keel, R. T. Doty, Z. Yang, J. G. Quigley, J. Chen, S. Knoblaugh, P. D. Kingsley, I. De Domenico, M. B. Vaughn, J. Kaplan, J. Palis and J. L. Abkowitz, *Science*, 2008, **319**, 825-828.

91. D. Finazzi and P. Arosio, *Arch Toxicol*, 2014, **88**, 1787-1802.

92. S. Leidgens, K. Z. Bullough, H. Shi, F. Li, M. Shakoury-Elizeh, T. Yabe, P. Subramanian, E. Hsu, N. Natarajan, A. Nandal, T. L. Stemmler and C. C. Philpott, *J Biol Chem*, 2013, **288**, 17791-17802.

93. S. Levi, B. Corsi, M. Bosisio, R. Invernizzi, A. Volz, D. Sanford, P. Arosio and J. Drysdale, *J Biol Chem*, 2001, **276**, 24437-24440.

94. P. Santambrogio, G. Biasiotto, F. Sanvito, S. Olivieri, P. Arosio and S. Levi, *J Histochem Cytochem*, 2007, **55**, 1129-1137.

95. in *Brenner & Rector's The Kidney*, eds. M. W. Taal, G. M. Chertow, P. A. Marsden, K. Skorecki, A. S. L. Yu and B. M. Brenner, Saunders, Philadelphia, 9 edn., 2012, pp. 1-3064.

96. in *Seldin and Giebisch's The Kidney: Physiology & Pathophysiology*, eds. R. J. Alpern, M. J. Caplan and O. W. Moe, Academic Press, London, 5 edn., 2013, pp. 1-3352.

Metallomics Accepted Manuscript

97. in *Handbook of Physiology*, ed. E. E. Windhager, Oxford University Press, Oxford, 1992, pp. 1-2544.

98. W. D. Comper, B. Haraldsson and W. M. Deen, *J Am Soc Nephrol*, 2008, **19**, 427-432.

99. E. I. Christensen and H. Birn, *Nat Rev Mol Cell Biol*, 2002, **3**, 256-266.

100. C. D. Klaassen, J. Liu and B. A. Diwan, *Toxicol Appl Pharmacol*, 2009, **238**, 215-220.

101. M. J. Burne, T. M. Osicka and W. D. Comper, *Kidney Int*, 1999, **55**, 261-270.

102. P. R. Cutillas, R. J. Chalkley, K. C. Hansen, R. Cramer, A. G. Norden, M. D. Waterfield, A. L. Burlingame and R. J. Unwin, *Am J Physiol Renal Physiol*, 2004, **287**, F353-364.

103. D. Zhang, E. Meyron-Holtz and T. A. Rouault, *J Am Soc Nephrol*, 2007, **18**, 401-406.

104. P. Aisen, in *Iron metabolism in health and disease*, eds. J. H. Brock, J. W. Halliday, M. J. Pippard and L. W. Powell, W. B. Saunders, London, 1994, pp. 1-30.

105. L. Kjeldsen, A. H. Johnsen, H. Sengelov and N. Borregaard, *J Biol Chem*, 1993, **268**, 10425-10432.

106. A. Tojo and S. Kinugasa, *Int J Nephrol*, 2012, **2012**, 481520.

107. E. D. Klootwijk, M. Reichold, R. J. Unwin, R. Kleta, R. Warth and D. Bockenhauer, *Nephrol Dial Transplant*, 2015, **30**, 1456-1460.

108. E. I. Christensen, H. Birn, T. Storm, K. Weyer and R. Nielsen, *Physiology (Bethesda)*, 2012, **27**, 223-236.

109. B. Seetharam, E. I. Christensen, S. K. Moestrup, T. G. Hammond and P. J. Verroust, *J Clin Invest*, 1997, **99**, 2317-2322.

110. J. C. Fyfe, M. Madsen, P. Hojrup, E. I. Christensen, S. M. Tanner, A. de la Chapelle, Q. He and S. K. Moestrup, *Blood*, 2004, **103**, 1573-1579.

111. S. Amsellem, J. Gburek, G. Hamard, R. Nielsen, T. E. Willnow, O. Devuyst, E. Nexo, P. J. Verroust, E. I. Christensen and R. Kozyraki, *J Am Soc Nephrol*, 2010, **21**, 1859-1867.

112. V. Hvidberg, C. Jacobsen, R. K. Strong, J. B. Cowland, S. K. Moestrup and N. Borregaard, *FEBS Lett*, 2005, **579**, 773-777.

113. S. Cui, P. J. Verroust, S. K. Moestrup and E. I. Christensen, *Am J Physiol*, 1996, **271**, F900-907.

Metallomics Accepted Manuscript

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43
44
45
46
47
48
49
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53
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55
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57
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59
60

114. H. P. Peters, C. M. Laarakkers, P. Pickkers, R. Masereeuw, O. C. Boerman, A. Eek, E. A. Cornelissen, D. W. Swinkels and J. F. Wetzels, *BMC Nephrol*, 2013, **14**, 70.

115. H. Birn, J. C. Fyfe, C. Jacobsen, F. Mounier, P. J. Verroust, H. Orskov, T. E. Willnow, S. K. Moestrup and E. I. Christensen, *J Clin Invest*, 2000, **105**, 1353-1361.

116. N. E. Magnusson, M. Hornum, K. A. Jorgensen, J. M. Hansen, C. Bistrup, B. Feldt-Rasmussen and A. Flyvbjerg, *BMC Nephrol*, 2012, **13**, 8.

117. R. A. Orlando, M. Exner, R. P. Czekay, H. Yamazaki, A. Saito, R. Ullrich, D. Kerjaschki and M. G. Farquhar, *Proc Natl Acad Sci U S A*, 1997, **94**, 2368-2373.

118. R. M. Sandoval, M. C. Wagner, M. Patel, S. B. Campos-Bilderback, G. J. Rhodes, E. Wang, S. E. Wean, S. S. Clendenon and B. A. Molitoris, *J Am Soc Nephrol*, 2012, **23**, 447-457.

119. V. Tenten, S. Menzel, U. Kunter, E. M. Sicking, C. R. van Roeyen, S. K. Sanden, M. Kaldenbach, P. Boor, A. Fuss, S. Uhlig, R. Lanzmich, B. Willemsen, H. Dijkman, M. Grepl, K. Wild, W. Kriz, B. Smeets, J. Floege and M. J. Moeller, *J Am Soc Nephrol*, 2013, **24**, 1966-1980.

120. E. I. Christensen and H. Birn, *Nat Rev Nephrol*, 2013, **9**, 700-702.

121. L. E. Dickson, M. C. Wagner, R. M. Sandoval and B. A. Molitoris, *J Am Soc Nephrol*, 2014, **25**, 443-453.

122. S. Cui, S. Nielsen, T. Bjerke and E. I. Christensen, *Exp Nephrol*, 1993, **1**, 309-318.

123. B. Moulouel, D. Houamel, C. Delaby, D. Tchernitchko, S. Vaulont, P. Letteron, O. Thibaudeau, H. Puy, L. Gouya, C. Beaumont and Z. Karim, *Kidney Int*, 2013, **84**, 756-766.

124. H. Tsunoo and H. H. Sussman, *J Biol Chem*, 1983, **258**, 4118-4122.

125. H. Fuchs and R. Gessner, *Biochim Biophys Acta*, 2002, **1570**, 19-26.

126. M. Haley, M. Lowe and C. P. Smith, *Proc Physiol Soc*, 2012, **27**, 24P (abstract C41).

127. B. Mackenzie, M. L. Ujwal, M. H. Chang, M. F. Romero and M. A. Hediger, *Pflugers Arch*, 2006, **451**, 544-558.

128. M. A. Su, C. C. Trenor, J. C. Fleming, M. D. Fleming and N. C. Andrews, *Blood*, 1998, **92**, 2157-2163.

Metallomics Accepted Manuscript

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129. P. L. Lee, T. Gelbart, C. West, C. Halloran and E. Beutler, *Blood Cells Mol Dis*, 1998, **24**, 199-215.

130. N. Hubert and M. W. Hentze, *Proc Natl Acad Sci U S A*, 2002, **99**, 12345-12350.

131. B. Mackenzie, H. Takanaga, N. Hubert, A. Rolfs and M. A. Hediger, *Biochem J*, 2007, **403**, 59-69.

132. M. Okubo, K. Yamada, M. Hosoyamada, T. Shibasaki and H. Endou, *Toxicol Appl Pharmacol*, 2003, **187**, 162-167.

133. M. Arredondo, M. J. Mendiburo, S. Flores, S. T. Singleton and M. D. Garrick, *Biometals*, 2014, **27**, 115-123.

134. N. Jabado, F. Canonne-Hergaux, S. Gruenheid, V. Picard and P. Gros, *Blood*, 2002, **100**, 2617-2622.

135. M. Tabuchi, T. Yoshimori, K. Yamaguchi, T. Yoshida and F. Kishi, *J Biol Chem*, 2000, **275**, 22220-22228.

136. M. Tabuchi, N. Tanaka, J. Nishida-Kitayama, H. Ohno and F. Kishi, *Mol Biol Cell*, 2002, **13**, 4371-4387.

137. M. Abouhamed, J. Gburek, W. Liu, B. Torchalski, A. Wilhelm, N. A. Wolff, E. I. Christensen, F. Thévenod and C. P. Smith, *Am J Physiol Renal Physiol*, 2006, **290**, F1525-F1533.

138. F. Canonne-Hergaux and P. Gros, *Kidney Int*, 2002, **62**, 147-156.

139. S. Lam-Yuk-Tseung and P. Gros, *Biochemistry*, 2006, **45**, 2294-2301.

140. T. Veuthey, M. C. D'Anna and M. E. Roque, *Am J Physiol Renal Physiol*, 2008, **295**, F1213-F1221.

141. P. Aisen, A. Leibman and J. Zweier, *J Biol Chem*, 1978, **253**, 1930-1937.

142. S. Haldar, A. Tripathi, J. Qian, A. Beserra, S. Suda, M. McElwee, J. Turner, U. Hopfer and N. Singh, *J Biol Chem*, 2015, **290**, 5512-5522.

143. N. A. Wolff, M. D. Garrick, L. M. Garrick and F. Thévenod, *Acta Physiol (Oxf)*, 2015, **213 (Suppl 699)**, 83 (P039).

144. A. C. Chua and E. H. Morgan, *J Comp Physiol B*, 1997, **167**, 361-369.

Metallomics Accepted Manuscript

1
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42
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44
45
46
47
48
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55
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57
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60

145. C. J. Ferguson, M. Wareing, M. Delannoy, R. Fenton, S. J. McLarnon, N. Ashton, A. G. Cox, R. F. McMahon, L. M. Garrick, R. Green, C. P. Smith and D. Riccardi, *Kidney Int*, 2003, **64**, 1755-1764.

146. T. Veuthey, D. Hoffmann, V. S. Vaidya and M. Wessling-Resnick, *Am J Physiol Renal Physiol*, 2014, **306**, F333-343.

147. S. Jenkitkasemwong, C. Y. Wang, B. Mackenzie and M. D. Knutson, *Biometals*, 2012, **25**, 643-655.

148. L. He, K. Girijashanker, T. P. Dalton, J. Reed, H. Li, M. Soleimani and D. W. Nebert, *Mol Pharmacol*, 2006, **70**, 171-180.

149. K. Girijashanker, L. He, M. Soleimani, J. M. Reed, H. Li, Z. Liu, B. Wang, T. P. Dalton and D. W. Nebert, *Mol Pharmacol*, 2008, **73**, 1413-1423.

150. J. P. Liuzzi, F. Aydemir, H. Nam, M. D. Knutson and R. J. Cousins, *Proc Natl Acad Sci U S A*, 2006, **103**, 13612-13617.

151. J. J. Pinilla-Tenas, B. K. Sparkman, A. Shawki, A. C. Illing, C. J. Mitchell, N. Zhao, J. P. Liuzzi, R. J. Cousins, M. D. Knutson and B. Mackenzie, *Am J Physiol Cell Physiol*, 2011, **301**, C862-871.

152. C. Y. Wang, S. Jenkitkasemwong, S. Duarte, B. K. Sparkman, A. Shawki, B. Mackenzie and M. D. Knutson, *J Biol Chem*, 2012, **287**, 34032-34043.

153. B. Wang, S. N. Schneider, N. Dragin, K. Girijashanker, T. P. Dalton, L. He, M. L. Miller, K. F. Stringer, M. Soleimani, D. D. Richardson and D. W. Nebert, *Am J Physiol Cell Physiol*, 2007, **292**, C1523-C1535.

154. A. Lymboussaki, E. Pignatti, G. Montosi, C. Garuti, D. J. Haile and A. Pietrangelo, *J Hepatol*, 2003, **39**, 710-715.

155. N. Wilkinson and K. Pantopoulos, *Front Pharmacol*, 2014, **5**, 176.

156. D. L. Zhang, R. M. Hughes, H. Ollivierre-Wilson, M. C. Ghosh and T. A. Rouault, *Cell Metab*, 2009, **9**, 461-473.

157. B. K. Fuqua, Y. Lu, D. Darshan, D. M. Frazer, S. J. Wilkins, N. Wolkow, A. G. Bell, J. Hsu, C. C. Yu, H. Chen, J. L. Dunaief, G. J. Anderson and C. D. Vulpe, *PLoS One*, 2014, **9**, e98792.

Metallomics Accepted Manuscript

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42
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46
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158. I. De Domenico, D. M. Ward, M. C. di Patti, S. Y. Jeong, S. David, G. Musci and J. Kaplan, *Embo J*, 2007, **26**, 2823-2831.

159. D. M. Ward and J. Kaplan, *Biochim Biophys Acta*, 2012, **1823**, 1426-1433.

160. N. A. Wolff, W. Liu, R. A. Fenton, W. K. Lee, F. Thévenod and C. P. Smith, *J Cell Mol Med*, 2011, **15**, 209-219.

161. R. R. Starzynski, F. Canonne-Hergaux, M. Lenartowicz, W. Krzeptowski, A. Willemetz, A. Stys, J. Bierla, P. Pietrzak, T. Dziaman and P. Lipinski, *Biochem J*, 2013, **449**, 69-78.

162. A. Zarjou, S. Bolisetty, R. Joseph, A. Traylor, E. O. Apostolov, P. Arosio, J. Balla, J. Verlander, D. Darshan, L. C. Kuhn and A. Agarwal, *J Clin Invest*, 2013, **123**, 4423-4434.

163. Y. Scindia, P. Dey, A. Thirunagari, H. Liping, D. L. Rosin, M. Floris, M. D. Okusa and S. Swaminathan, *J Am Soc Nephrol*, 2015, DOI: 10.1681/ASN.2014101037.

164. J. G. Hoenderop, D. Muller, A. W. Van Der Kemp, A. Hartog, M. Suzuki, K. Ishibashi, M. Imai, F. Sweep, P. H. Willems, C. H. Van Os and R. J. Bindels, *J Am Soc Nephrol*, 2001, **12**, 1342-1349.

165. R. Vennekens, J. G. Hoenderop, J. Prenen, M. Stuiver, P. H. Willems, G. Droogmans, B. Nilius and R. J. Bindels, *J Biol Chem*, 2000, **275**, 3963-3969.

166. B. Nilius, R. Vennekens, J. Prenen, J. G. Hoenderop, R. J. Bindels and G. Droogmans, *J Physiol*, 2000, **527 Pt 2**, 239-248.

167. M. van Abel, J. G. Hoenderop, A. W. van der Kemp, M. M. Friedlaender, J. P. van Leeuwen and R. J. Bindels, *Kidney Int*, 2005, **68**, 1708-1721.

168. T. de Groot, R. J. Bindels and J. G. Hoenderop, *Kidney Int*, 2008, **74**, 1241-1246.

169. A. Qiu and C. Hogstrand, *Gene*, 2004, **342**, 113-123.

170. E. Perez-Reyes, *Physiol Rev*, 2003, **83**, 117-161.

171. W. A. Catterall, *Cold Spring Harb Perspect Biol*, 2011, **3**, a003947.

172. D. M. Rodman, K. Reese, J. Harral, B. Fouty, S. Wu, J. West, M. Hoedt-Miller, Y. Tada, K. X. Li, C. Cool, K. Fagan and L. Cribbs, *Circ Res*, 2005, **96**, 864-872.

173. A. M. Yunker and M. W. McEnery, *J Bioenerg Biomembr*, 2003, **35**, 533-575.

Metallomics Accepted Manuscript

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174. P. B. Hansen, *Am J Physiol Regul Integr Comp Physiol*, 2015, **308**, R227-237.

175. D. Andreasen, B. L. Jensen, P. B. Hansen, T. H. Kwon, S. Nielsen and O. Skott, *Am J Physiol Renal Physiol*, 2000, **279**, F997-F1005.

176. M. Leclerc, M. G. Brunette and D. Couchourel, *Kidney Int*, 2004, **66**, 242-250.

177. M. G. Brunette, M. Leclerc, D. Couchourel, J. Mailloux and Y. Bourgeois, *Can J Physiol Pharmacol*, 2004, **82**, 30-37.

178. D. Couchourel, M. Leclerc, J. Filep and M. G. Brunette, *Mol Cell Endocrinol*, 2004, **222**, 71-81.

179. S. Kumfu, S. Chattipakorn, S. Srichairatanakool, J. Settakorn, S. Fucharoen and N. Chattipakorn, *Eur J Haematol*, 2011, **86**, 156-166.

180. K. V. Lopin, I. P. Gray, C. A. Obejero-Paz, F. Thévenod and S. W. Jones, *Mol Pharmacol*, 2012, **82**, 1194-1204.

181. K. V. Lopin, C. A. Obejero-Paz and S. W. Jones, *J Membr Biol*, 2010, **235**, 131-143.

182. C. Langelueddecke, E. Roussa, R. A. Fenton, N. A. Wolff, W. K. Lee and F. Thévenod, *J Biol Chem*, 2012, **287**, 159-169.

183. N. Paragas, R. Kulkarni, M. Werth, K. M. Schmidt-Ott, C. Forster, R. Deng, Q. Zhang, E. Singer, A. D. Klose, T. H. Shen, K. P. Francis, S. Ray, S. Vijayakumar, S. Seward, M. E. Bovino, K. Xu, Y. Takabe, F. E. Amaral, S. Mohan, R. Wax, K. Corbin, S. Sanna-Cherchi, K. Mori, L. Johnson, T. Nickolas, V. D'Agati, C. S. Lin, A. Qiu, Q. Al-Awqati, A. J. Ratner and J. Barasch, *J Clin Invest*, 2014, **124**, 2963-2976.

184. B. S. Nielsen, N. Borregaard, J. R. Bundgaard, S. Timshel, M. Sehested and L. Kjeldsen, *Gut*, 1996, **38**, 414-420.

185. D. H. Goetz, M. A. Holmes, N. Borregaard, M. E. Bluhm, K. N. Raymond and R. K. Strong, *Mol Cell*, 2002, **10**, 1033-1043.

186. G. Bao, M. Clifton, T. M. Hoette, K. Mori, S. X. Deng, A. Qiu, M. Viltard, D. Williams, N. Paragas, T. Leete, R. Kulkarni, X. Li, B. Lee, A. Kalandadze, A. J. Ratner, J. C. Pizarro, K. M. Schmidt-Ott, D. W. Landry, K. N. Raymond, R. K. Strong and J. Barasch, *Nat Chem Biol*, 2010, **6**, 602-609.

Metallomics Accepted Manuscript

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57
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60

187. T. H. Flo, K. D. Smith, S. Sato, D. J. Rodriguez, M. A. Holmes, R. K. Strong, S. Akira and A. Aderem, *Nature*, 2004, **432**, 917-921.

188. T. Berger, A. Togawa, G. S. Duncan, A. J. Elia, A. You-Ten, A. Wakeham, H. E. Fong, C. C. Cheung and T. W. Mak, *Proc Natl Acad Sci U S A*, 2006, **103**, 1834-1839.

189. R. J. Abergel, M. C. Clifton, J. C. Pizarro, J. A. Warner, D. K. Shuh, R. K. Strong and K. N. Raymond, *J Am Chem Soc*, 2008, **130**, 11524-11534.

190. J. Yang, K. Mori, J. Y. Li and J. Barasch, *Am J Physiol Renal Physiol*, 2003, **285**, F9-18.

191. K. M. Schmidt-Ott, K. Mori, J. Y. Li, A. Kalandadze, D. J. Cohen, P. Devarajan and J. Barasch, *J Am Soc Nephrol*, 2007, **18**, 407-413.

192. A. Haase-Fielitz, M. Haase and P. Devarajan, *Ann Clin Biochem*, 2014, **51**, 335-351.

193. W. F. t. Peacock, A. Maisel, J. Kim and C. Ronco, *Postgrad Med*, 2013, **125**, 82-93.

194. N. Paragas, A. Qiu, Q. Zhang, B. Samstein, S. X. Deng, K. M. Schmidt-Ott, M. Viltard, W. Yu, C. S. Forster, G. Gong, Y. Liu, R. Kulkarni, K. Mori, A. Kalandadze, A. J. Ratner, P. Devarajan, D. W. Landry, V. D'Agati, C. S. Lin and J. Barasch, *Nat Med*, 2011, **17**, 216-222.

195. J. Mishra, Q. Ma, A. Prada, M. Mitsnefes, K. Zahedi, J. Yang, J. Barasch and P. Devarajan, *J Am Soc Nephrol*, 2003, **14**, 2534-2543.

196. L. R. Devireddy, C. Gazin, X. Zhu and M. R. Green, *Cell*, 2005, **123**, 1293-1305.

197. L. R. Devireddy, J. G. Teodoro, F. A. Richard and M. R. Green, *Science*, 2001, **293**, 829-834.

198. H. Koepsell and H. Endou, *Pflugers Arch*, 2004, **447**, 666-676.

199. K. M. Bennett, J. Liu, C. Hoelting and J. Stoll, *Mol Cell Biochem*, 2011, **352**, 143-154.

200. W. K. Fang, L. Y. Xu, X. F. Lu, L. D. Liao, W. J. Cai, Z. Y. Shen and E. M. Li, *Biochem J*, 2007, **403**, 297-303.

201. C. J. Wienken, P. Baaske, U. Rothbauer, D. Braun and S. Duhr, *Nat Commun*, 2010, **1**, 100.

202. A. Tojo and H. Endou, *Am J Physiol*, 1992, **263**, F601-F606.

203. P. D. Leber and D. J. Marsh, *Am J Physiol*, 1970, **219**, 358-363.

204. R. G. Galaske, J. B. Van Liew and L. G. Feld, *Kidney Int*, 1979, **16**, 394-403.

205. K. M. Madsen, R. H. Harris and C. C. Tisher, *Kidney Int*, 1982, **21**, 354-361.

Metallomics Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

206. E. I. Christensen, P. J. Verroust and R. Nielsen, *Pflugers Arch*, 2009, **458**, 1039-1048.

207. R. B. Klassen, K. Crenshaw, R. Kozyraki, P. J. Verroust, L. Tio, S. Atrian, P. L. Allen and T. G. Hammond, *Am J Physiol Renal Physiol*, 2004, **287**, F393-F403.

208. D. F. Akintola, B. Sampson and A. Fleck, *J Lab Clin Med*, 1995, **126**, 119-127.

209. H. Milnerowicz and A. Bizon, *Acta Biochim Pol*, 2010, **57**, 99-104.

210. R. Nielsen and E. I. Christensen, *Pediatr Nephrol*, 2010, **25**, 813-822.

211. C. Kastner, M. Pohl, M. Sendeski, G. Stange, C. A. Wagner, B. Jensen, A. Patzak, S. Bachmann and F. Theilig, *Am J Physiol Renal Physiol*, 2009, **296**, F902-F911.

212. A. C. Alfrey and W. S. Hammond, *Kidney Int*, 1990, **37**, 1409-1413.

213. N. Ishizaka, K. Saito, E. Noiri, M. Sata, I. Mori, M. Ohno and R. Nagai, *Nephron Physiol*, 2004, **98**, p107-113.

214. E. Dizin, U. Hasler, S. Nlandu-Khodo, M. Fila, I. Roth, T. Ernandez, A. Doucet, P. Y. Martin, E. Feraille and S. de Seigneux, *Am J Physiol Renal Physiol*, 2013, **305**, F1053-F1063.

215. F. Thévenod and W. K. Lee, *Met Ions Life Sci*, 2013, **11**, 415-490.

216. J. M. Moulis, *Biometals*, 2010, **23**, 877-896.

217. T. S. Nawrot, J. A. Staessen, H. A. Roels, E. Munters, A. Cuypers, T. Richart, A. Ruttens, K. Smeets, H. Clijsters and J. Vangronsveld, *Biometals*, 2010, **23**, 769-782.

218. A. Cuypers, M. Plusquin, T. Remans, M. Jozefczak, E. Keunen, H. Gielen, K. Opdenakker, A. R. Nair, E. Munters, T. J. Artois, T. Nawrot, J. Vangronsveld and K. Smeets, *Biometals*, 2010, **23**, 927-940.

219. F. Thévenod and W. K. Lee, *Arch Toxicol*, 2013, **87**, 1743-1786.

220. W. Maret and J. M. Moulis, *Met Ions Life Sci*, 2013, **11**, 1-29.

221. C. C. Bridges and R. K. Zalups, *Toxicol Appl Pharmacol*, 2005, **204**, 274-308.

222. R. Cornelis, B. Heinzow, R. F. Herber, J. M. Christensen, O. M. Poulsen, E. Sabbioni, D. M. Templeton, Y. Thomassen, M. Vahter and O. Vesterberg, *J Trace Elem Med Biol*, 1996, **10**, 103-127.

223. M. Nordberg and G. F. Nordberg, *Environ Health Perspect*, 1975, **12**, 103-108.

Metallomics Accepted Manuscript

224. R. E. Dudley, L. M. Gammal and C. D. Klaassen, *Toxicol Appl Pharmacol*, 1985, **77**, 414-426.

225. K. Nomiyama, H. Nomiyama and N. Kameda, *Toxicology*, 1998, **129**, 157-168.

226. S. Thijssen, J. Maringwa, C. Faes, I. Lambrichts and E. Van Kerkhove, *Toxicology*, 2007, **229**, 145-156.

227. L. Jarup, A. Rogenfelt, C. G. Elinder, K. Nogawa and T. Kjellstrom, *Scand J Work Environ Health*, 1983, **9**, 327-331.

228. K. Klotz, W. Weistenhofer and H. Drexler, *Met Ions Life Sci*, 2013, **11**, 85-98.

229. E. Freisinger and M. Vasak, *Met Ions Life Sci*, 2013, **11**, 339-371.

230. H. M. Chan, Y. Tamura, M. G. Cherian and R. A. Goyer, *Proc Soc Exp Biol Med*, 1993, **202**, 420-427.

231. K. Tanaka, *Dev Toxicol Environ Sci*, 1982, **9**, 237-249.

232. S. A. Gunn and T. C. Gould, *Proc Soc Exp Biol Med*, 1957, **96**, 820-823.

233. G. F. Nordberg and K. Nishiyama, *Arch Environ Health*, 1972, **24**, 209-214.

234. H. M. Chan, L. F. Zhu, R. Zhong, D. Grant, R. A. Goyer and M. G. Cherian, *Toxicol Appl Pharmacol*, 1993, **123**, 89-96.

235. I. Sabolic, D. Breljak, M. Skarica and C. M. Herak-Kramberger, *Biometals*, 2010, **23**, 897-926.

236. W. Maret, *J Biol Inorg Chem*, 2011, **16**, 1079-1086.

237. T. Kjellstrom, *Environ Health Perspect*, 1979, **28**, 169-197.

238. H. Roels, R. Lauwerys, J. P. Buchet, A. Bernard, J. S. Garvey and H. J. Linton, *Int Arch Occup Environ Health*, 1983, **52**, 159-166.

239. C. Tohyama, Z. A. Shaikh, K. Nogawa, E. Kobayashi and R. Honda, *Toxicology*, 1981, **20**, 289-297.

240. W. C. Prozialeck and J. R. Edwards, *Biometals*, 2010, **23**, 793-809.

241. L. Jarup, *Nephrol Dial Transplant*, 2002, **17 Suppl 2**, 35-39.

242. H. Roels, A. Bernard, J. P. Buchet, A. Goret, R. Lauwerys, D. R. Chettle, T. C. Harvey and I. A. Haddad, *Lancet*, 1979, **1**, 221.

243. WHO, *Cadmium*, World Health Organization, 1992.

Metallomics Accepted Manuscript

1
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3
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5
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7
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9
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54
55
56
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59
60

244. Y. Suzuki, *J Toxicol Environ Health*, 1980, **6**, 469-482.

245. P. M. Ferraro, S. Costanzi, A. Naticchia, A. Sturniolo and G. Gambaro, *BMC Public Health*, 2010, **10**, 304.

246. A. Navas-Acien, M. Tellez-Plaza, E. Guallar, P. Muntner, E. Silbergeld, B. Jaar and V. Weaver, *Am J Epidemiol*, 2009, **170**, 1156-1164.

247. A. Hartwig, *Met Ions Life Sci*, 2013, **11**, 491-507.

248. A. Bernard, J. P. Buchet, H. Roels, P. Masson and R. Lauwerys, *Eur J Clin Invest*, 1979, **9**, 11-22.

249. K. Nogawa, A. Ishizaki, M. Fukushima, I. Shibata and N. Hagino, *Environ Res*, 1975, **10**, 280-307.

250. N. Johri, G. Jacquillet and R. Unwin, *Biometals*, 2010, **23**, 783-792

251. W. Goumakos, J. P. Laussac and B. Sarkar, *Biochem Cell Biol*, 1991, **69**, 809-820.

252. W. R. Harris and L. J. Madsen, *Biochemistry*, 1988, **27**, 284-288.

253. D. J. Price and J. G. Joshi, *J Biol Chem*, 1983, **258**, 10873-10880.

254. T. Granier, G. Comberton, B. Gallois, B. L. d'Estaintot, A. Dautant, R. R. Crichton and G. Precigoux, *Proteins*, 1998, **31**, 477-485.

255. W. C. Prozialeck and P. C. Lamar, *Arch Toxicol*, 1993, **67**, 113-119.

256. R. K. Zalups, *Toxicol Appl Pharmacol*, 2000, **164**, 15-23.

257. S. Soodvilai, J. Nantavishit, C. Muanprasat and V. Chatsudthipong, *Toxicol Lett*, 2011, **204**, 38-42.

258. F. Thévenod, G. Ciarimboli, M. Leistner, N. A. Wolff, W. K. Lee, I. Schatz, T. Keller, R. Al-Monajjed, V. Gorboulev and H. Koepsell, *Mol Pharm*, 2013, **10**, 3045-3056.

259. C. D. Klaassen, J. Liu and S. Choudhuri, *Annu Rev Pharmacol Toxicol*, 1999, **39**, 267-294.

260. N. A. Wolff, M. Abouhamed, P. J. Verroust and F. Thévenod, *J Pharmacol Exp Ther*, 2006, **318**, 782-791.

261. N. A. Wolff, W. K. Lee, M. Abouhamed and F. Thévenod, *Toxicol. Appl. Pharmacol.*, 2008, **230**, 78-85.

262. C. Erfurt, E. Roussa and F. Thévenod, *Am J Physiol Cell Physiol*, 2003, **285**, C1367-C1376.

Metallomics Accepted Manuscript

263. N. A. Wolff, W. K. Lee and F. Thévenod, *Toxicol Lett*, 2011, **203**, 210-218.

264. M. Abouhamed, N. A. Wolff, W. K. Lee, C. P. Smith and F. Thévenod, *Am J Physiol Renal Physiol*, 2007, **293**, F705-F712.

265. R. D. Palmiter, *Proc Natl Acad Sci U S A*, 1994, **91**, 1219-1223.

266. Y. Liu, J. Liu, S. M. Habeebu, M. P. Waalkes and C. D. Klaassen, *Toxicol Sci*, 2000, **57**, 167-176.

267. C. Dorian, V. H. Gattone, 2nd and C. D. Klaassen, *Toxicol Appl Pharmacol*, 1992, **114**, 173-181.

268. C. Dorian, V. H. Gattone, 2nd and C. D. Klaassen, *Toxicol Appl Pharmacol*, 1992, **117**, 242-248.

269. W. Tang and Z. A. Shaikh, *J Toxicol Environ Health A*, 2001, **63**, 221-235.

270. M. Nordberg, *Environ Health Perspect*, 1984, **54**, 13-20.

271. J. Sudo, T. Hayashi, J. Terui, M. Soyama, M. Fukata and K. Kakuno, *Eur J Pharmacol*, 1994, **270**, 229-235.

272. A. Onodera, M. Tani, T. Michigami, M. Yamagata, K. S. Min, K. Tanaka, T. Nakanishi, T. Kimura and N. Itoh, *Toxicol Lett*, 2012, **212**, 91-96.

273. E. Felley-Bosco and J. Diezi, *Toxicol Appl Pharmacol*, 1987, **91**, 204-211.

274. F. Thévenod, *Nephron Physiol*, 2003, **93**, 87-93.

275. A. Chaumont, M. Nickmilder, X. Dumont, T. Lundh, S. Skerfving and A. Bernard, *Toxicol Lett*, 2012, **210**, 345-352.

276. P. E. Evrin and L. Wibell, *Scand J Clin Lab Invest*, 1972, **29**, 69-74.

277. K. Takagi, K. Kin, Y. Itoh, H. Enomoto and T. Kawai, *J Clin Pathol*, 1980, **33**, 786-791.

278. J. R. Leheste, B. Rolinski, H. Vorum, J. Hilpert, A. Nykjaer, C. Jacobsen, P. Aucouturier, J. O. Moskaug, A. Otto, E. I. Christensen and T. E. Willnow, *Am J Pathol*, 1999, **155**, 1361-1370.

279. C. M. Eakin, J. D. Knight, C. J. Morgan, M. A. Gelfand and A. D. Miranker, *Biochemistry*, 2002, **41**, 10646-10656.

280. A. G. Norden, P. Sharratt, P. R. Cutillas, R. Cramer, S. C. Gardner and R. J. Unwin, *Kidney Int*, 2004, **66**, 1994-2003.

281. H. Birn and E. I. Christensen, *Kidney Int*, 2006, **69**, 440-449.

Metallomics Accepted Manuscript

282. J. Liu, R. A. Goyer and M. P. Waalkes, in *Casarett and Doull's Toxicology*, ed. C. D. Klaassen, McGraw-Hill, New York, NY, 5th edn., 2008, pp. 931-979.

283. L. Jarup, B. Persson and C. G. Elinder, *Occup Environ Med*, 1995, **52**, 818-822.

284. A. Akesson, T. Lundh, M. Vahter, P. Bjellerup, J. Lidfeldt, C. Nerbrand, G. Samsioe, U. Stromberg and S. Skerfving, *Environ Health Perspect*, 2005, **113**, 1627-1631.

285. W. Xiao, Y. Liu and D. M. Templeton, *Toxicol Appl Pharmacol*, 2009, **238**, 315-326.

286. P. V. Rao, S. A. Jordan and M. K. Bhatnagar, *J Environ Pathol Toxicol Oncol*, 1989, **9**, 19-44.

287. J. P. Girolami, J. L. Bascands, C. Pecher, G. Cabos, J. P. Moatti, J. F. Mercier, J. M. Haguenoer and Y. Manuel, *Toxicology*, 1989, **55**, 117-129.

288. G. Oner, U. K. Senturk and N. Izgut-Uysal, *Biol Trace Elem Res*, 1995, **48**, 111-117.

289. R. Lauwerys and A. Bernard, *Toxicol Lett*, 1989, **46**, 13-29.

290. O. Barbier, G. Jacquillet, M. Tauc, P. Poujeol and M. Cougnon, *Am J Physiol Renal Physiol*, 2004, **287**, F1067-F1075.

291. L. Lacinova, N. Klugbauer and F. Hofmann, *Neuropharmacology*, 2000, **39**, 1254-1266.

292. D. Diaz, R. Bartolo, D. M. Delgadillo, F. Higueldo and J. C. Gomora, *J Membr Biol*, 2005, **207**, 91-105.

293. J. R. Serrano, E. Perez-Reyes and S. W. Jones, *J Gen Physiol*, 1999, **114**, 185-201.

294. E. M. Leslie, J. Liu, C. D. Klaassen and M. P. Waalkes, *Mol Pharmacol*, 2006, **69**, 629-639.

295. K. V. Lopin, F. Thévenod, J. C. Page and S. W. Jones, *Mol Pharmacol*, 2012, **82**, 1183-1193.

296. C. J. Frazier, E. G. George and S. W. Jones, *Biophys J*, 2000, **78**, 1872-1880.

297. G. Kovacs, N. Montalbetti, M. C. Franz, S. Graeter, A. Simonin and M. A. Hediger, *Cell Calcium*, 2013, **54**, 276-286.

298. E. K. Park, S. K. Mak and B. D. Hammock, *Interdiscip Toxicol*, 2013, **6**, 157-158.

299. C. Langelueddecke, W. K. Lee and F. Thévenod, *Toxicol Lett*, 2014, **226**, 228-235.

300. K. J. Ullrich, in *Kurzgefaßtes Lehrbuch der Physiologie*, ed. W. D. Keidel, Georg Thieme Verlag, Stuttgart, 1975, ch. 10.

Metallomics Accepted Manuscript

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301. M. Torra, J. To-Figueras, M. Brunet, M. Rodamilans and J. Corbella, *Bull Environ Contam Toxicol*, 1994, **53**, 509-515.

302. M. Yoshida, H. Ohta, Y. Yamauchi, Y. Seki, M. Sagi, K. Yamazaki and Y. Sumi, *Biol Trace Elem Res*, 1998, **63**, 167-175.

303. S. H. Garrett, M. A. Sens, J. H. Todd, S. Somji and D. A. Sens, *Toxicol Lett*, 1999, **105**, 207-214.

304. T. Nagamine, K. Nakazato, K. Suzuki, T. Kusakabe, T. Sakai, M. Oikawa, T. Satoh, T. Kamiya and K. Arakawa, *Biol Trace Elem Res*, 2007, **117**, 115-126.

305. L. Wang, D. Chen, H. Wang and Z. Liu, *Biol Trace Elem Res*, 2009, **129**, 190-199.

306. R. Campos, F. Maureira, A. Garrido and A. Valenzuela, *Comp Biochem Physiol B*, 1993, **105**, 157-163.

307. B. Gonzalez-Flecha, P. Evelson, N. Sterin-Speziale and A. Boveris, *Biochim Biophys Acta*, 1993, **1157**, 155-161.

308. A. P. Zou and A. W. Cowley, Jr., *Acta Physiol Scand*, 2003, **179**, 233-241.

309. R. A. Zager, A. C. Johnson and K. B. Frostad, *Am J Physiol Renal Physiol*, 2014, **307**, F856-868.

310. A. A. McDonough and S. C. Thompson, in *Brenner & Rector's The Kidney*, eds. M. W. Taal, G. M. Chertow, P. A. Marsden, K. Skorecki, A. S. L. Yu and B. M. Brenner, Saunders, Philadelphia, 9 edn., 2012, vol. 1, ch. 4, pp. 138-157.

311. A. M. Hall, G. J. Rhodes, R. M. Sandoval, P. R. Corridon and B. A. Molitoris, *Kidney Int*, 2013, **83**, 72-83.

312. A. Sharfuddin, S. D. Weisbord, P. M. Palevsky and B. A. Molitoris, in *Brenner & Rector's The Kidney*, eds. M. W. Taal, G. M. Chertow, P. A. Marsden, K. Skorecki, A. S. L. Yu and B. M. Brenner, Saunders, Philadelphia, 9 edn., 2012, vol. 1, ch. 30, pp. 1044-1099.

313. G. W. Richter, *J Exp Med*, 1957, **106**, 203-218.

314. S. Fagoonee, J. Gburek, E. Hirsch, S. Marro, S. K. Moestrup, J. M. Laurberg, E. I. Christensen, L. Silengo, F. Altruda and E. Tolosano, *Am J Pathol*, 2005, **166**, 973-983.

315. J. G. Wardeska, B. Viglione and N. D. Chasteen, *J Biol Chem*, 1986, **261**, 6677-6683.

316. J. H. Kagi and B. L. Vallee, *J Biol Chem*, 1961, **236**, 2435-2442.

Metallomics Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

317. X. Ding, E. Bill, M. Good, A. X. Trautwein and M. Vasak, *Eur J Biochem*, 1988, **171**, 711-714.

318. J. P. Muller, M. Vedel, M. J. Monnot, N. Touzet and M. Wegnez, *DNA Cell Biol*, 1991, **10**, 571-579.

319. D. M. Durnam and R. D. Palmiter, *Mol Cell Biol*, 1984, **4**, 484-491.

320. J. C. Fleet, G. K. Andrews and C. C. McCormick, *J Nutr*, 1990, **120**, 1214-1222.

321. J. H. Kagi and A. Schaffer, *Biochemistry*, 1988, **27**, 8509-8515.

322. D. Price and J. G. Joshi, *Proc Natl Acad Sci U S A*, 1982, **79**, 3116-3119.

323. W. K. Lee, U. Bork, F. Gholamrezaei and F. Thévenod, *Am J Physiol Renal Physiol*, 2005, **288**, F27-F39.

324. Y. Wang, J. Fang, S. S. Leonard and K. M. Rao, *Free Radic Biol Med*, 2004, **36**, 1434-1443.

325. W. K. Lee, M. Spielmann, U. Bork and F. Thévenod, *Am J Physiol Cell Physiol*, 2005, **289**, C656-C664.

326. W. K. Lee and F. Thévenod, *Am J Physiol Cell Physiol*, 2006, **291**, C195-C202.

327. M. Kippler, W. Goessler, B. Nermell, E. C. Ekstrom, B. Lonnerdal, S. El Arifeen and M. Vahter, *Environ Res*, 2009, **109**, 914-921.

328. K. Weyer, T. Storm, J. Shan, S. Vainio, R. Kozyraki, P. J. Verroust, E. I. Christensen and R. Nielsen, *Nephrol Dial Transplant*, 2011, **26**, 3446-3451.

14. Figure legends

Figure 1:

Structure and function of the nephron. For further details, see section *5.*

Metallomics Accepted Manuscript

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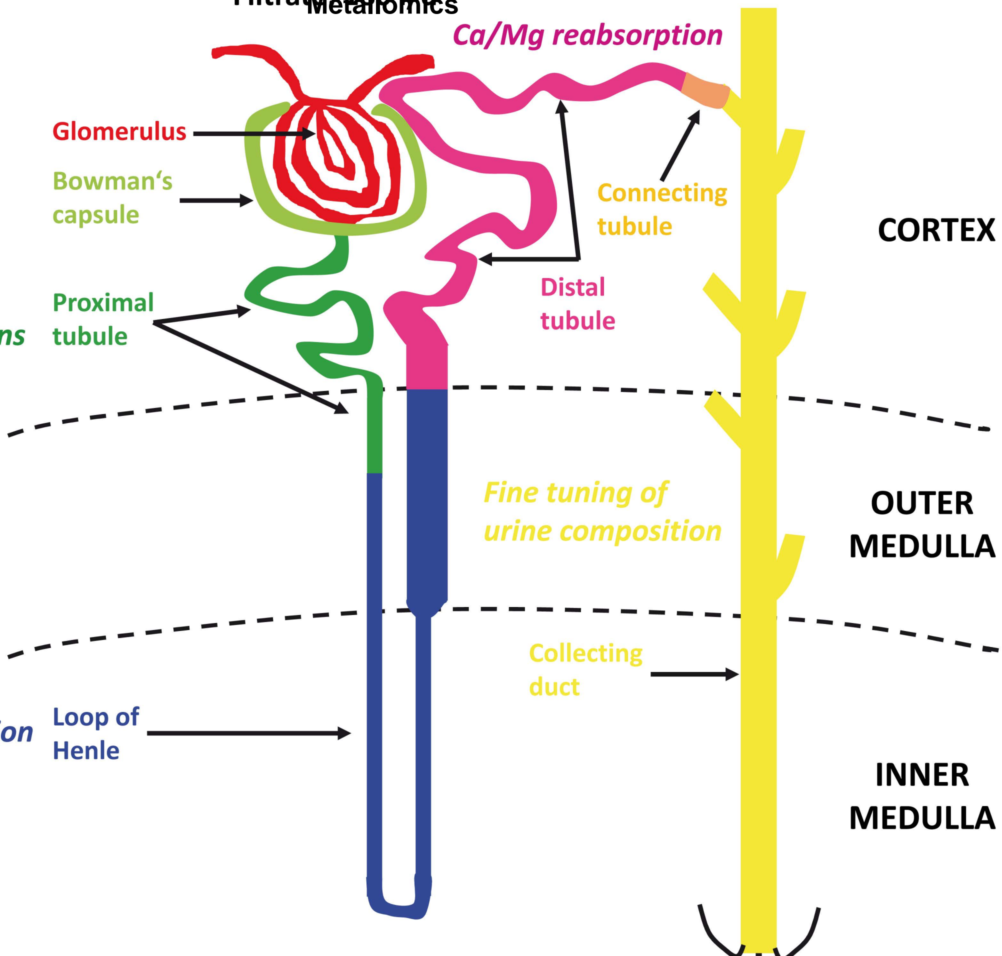

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Receptor/ Transporter	Nephron localization	Subcellular localization	Species	References	Comments
Megalin:cubilin: amnionless	PT*	apical; subapical	human; mouse; rat; rabbit	107	Immuno-fluorescence/-histochemistry/-gold; co-labeling with segment-/organelle-specific marker
Transferrin receptor 1	PT; CD	apical; subapical	mouse	102	Immunofluorescence; poor resolution; specificity of antibody unclear
NGAL**/24p3/lipocalin-2 receptor (SLC22A17)	DT; CD	apical; subapical	mouse; rat	181; 213	Immunofluorescence/-histochemistry; co-labeling with segment-specific marker
DMT1 (SLC11A2)	PT	Intracellular (endosomes/ lysosomes/ mitochondria)	mouse; rat	12; 14; 15; 84; 85; 136; 139	Immuno-fluorescence/-histochemistry/-gold; co-labeling with segment-/organelle-specific marker
		apical?	mouse	137	Immunohistochemistry; poor resolution; collapsed tubules; no co-labeling with segment-specific marker
	LOH	apical; intracellular	rat	14	Immunofluorescence; co-labeling with segment-/cell-specific marker
	DT	apical	rat	14; 15	Immunofluorescence; co-labeling with segment-/membrane-specific marker
		∅?	mouse	137; 139	Immunohistochemistry; poor resolution; collapsed tubules; no co-labeling with segment-specific marker; specificity of antibody unclear
	CD	apical; intracellular; basolateral	rat	14; 15; 144	Immunofluorescence; co-labeling with segment-/cell-specific marker
ZIP8 (SLC39A8)	PT	apical?; subapical	mouse	152	Immunofluorescence/-histochemistry; co-labeling with membrane-specific marker, yet poor resolution (discussed in [141])
ZIP14 (SLC39A14)	PT?	?	∅	∅	No staining of native tissue; staining in overexpressing cell lines only (discussed in [141])

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Ferroportin (FPN1/SLC40A1)	PT	basolateral	mouse; rat	122; 159; 160	Immuno-fluorescence/-histochemistry/-gold; co-labeling with segment-/membrane-specific marker
		apical/basolateral?	mouse	139; 161	Immunohistochemistry; poor resolution; no co-labeling with segment-specific marker; specificity of antibody unclear
	LOH	basolateral	mouse	122	Immunofluorescence; co-labeling with segment-/membrane-specific marker
		∅?	mouse	139	Immunohistochemistry; poor resolution; no co-labeling with segment-specific marker; specificity of antibody unclear
	CD	Intracellular?	mouse	139	Immunohistochemistry; poor resolution; no co-labeling with segment-specific marker; specificity of antibody unclear
		∅	mouse	122	Immunofluorescence; co-labeling with segment-/membrane-specific marker
TRPV5 (ECaC1)	DT	apical; subapical	rat	163	Immunofluorescence; co-labeling with segment-specific markers
$Ca_v3.1$ (α_{1G})	DT; CD	apical	rat	174	Immunohistochemistry; co-labeling with segment-specific markers

* PT: proximal tubule; LOH: loop of Henle; DT: distal tubule; CD: collecting duct.

** NGAL: neutrophil gelatinase-associated lipocalin.

Table 1: Localization and subcellular distribution of iron and cadmium transporting receptors, transporters and channels in the kidney

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		Fe			Cd^{2+}		
Uptake pathway	Localization	Subsrate/Ligand	$K_D/K_{0.5}$ (nmol/l)	References	Substrate/Ligand	$K_D/K_{0.5}$ (nmol/l)	References
Megalin:cubilin:amnionless	PT*	transferrin NGAL	20 60	16 111	?** Cd^{2+}-metallothionein	1000-100,000	206
Transferrin receptor 1	PT; CD	transferrin	0.2-0.4	123; 124	?	∅	∅
NGAL***/24p3/lipocalin-2 receptor (SLC22A17)	DT; CD	transferrin NGAL	100 0.090	181 196	Cd^{2+}-metallothionein	100	181
DMT1 (SLC11A2)	PT; LOH; DT; CD	Fe^{2+}	1000	11	Cd^{2+}	1000	11
ZIP8 (SLC39A8)	PT	Fe^{2+}	700	151	Cd^{2+}	620	147
ZIP14 (SLC39A14)	PT	Fe^{2+}	2300	150	Cd^{2+}	100-1100	148
Ferroportin (FPN1/SLC40A1)	PT; LOH; CD	Fe^{2+}	<100	41	∅	∅	41
TRPV5 (ECaC1)	DT	Fe^{2+}	<1000 (estimated)	168	Cd^{2+}	micromolar (estimated)	295
$Ca_v3.1$ (α_{1G})	DT; CD	Fe^{2+}	low micromolar (estimated)	179	Cd^{2+}	low nanomolar (estimated)	293

* PT: proximal tubule; LOH: loop of Henle; DT: distal tubule; CD: collecting duct.

** see section *8.2.* for detailed explanations.

*** NGAL: neutrophil gelatinase-associated lipocalin.

Table 2: Functional properties of iron and cadmium transporters receptors, transporters and channels of the kidney

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Megalin:cubilin:(amnionless)					
Ligand	**K_D (nmol/l)**	**References**	**Concentration in plasma (µmol/l)**	**References**	**Concentration in glomerular filtrate* (nmol/l)**
Transferrin	20	16	35	17	2
NGAL [human]/siderocalin/24p3 [rodent]/lipocalin-2	60	111	7	115	650
Albumin	630	114	690	17	53
Metallothionein	100,000	206	0.0005-0.005	207; 208	0.5-5
β2-microglobulin	420	277	0.11	17	100
α1-microglobulin	n.d.	107	1	17	92

* Calculations are based on estimated glomerular sieving coefficients of plasma proteins [17].

Table 3: Binding properties and estimated concentrations of ligands of megalin:cubilin in the glomerular filtrate of the kidney.

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