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Metal ion transporters and homeostasis

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Transition metals are essential for many metabolic processes and their homeostasis is crucial for life. Aberrations in the cellular metal ion concentrations may lead to cell death and severe diseases. Metal ion transporters play a major role in maintaining the correct concentrations of the various metal ions in the different cellular compartments. Recent studies of yeast mutants revealed key elements in metal ion homeostasis, including novel transport systems. Several of the proteins discovered in yeast are highly conserved, and defects in some of the yeast mutants could be complemented by their human homologs. The studies of yeast metal ion transporters helped to unravel the molecular mechanism of macrophage defense against bacterial infection and hereditary diseases.

Keywords: copper/human diseases/iron/manganese/metal ions/transport/yeast

Introduction

Metal ions are vital life elements that participate in numerous metabolic junctions in every living cell. Therefore, an aberration in metal ion homeostasis might cause death or severe illness. Metal ion homeostasis is maintained through highly regulated processes of uptake, storage and secretion. A specific set of transporters function in each cellular compartment to provide a delicate balance of transport activities across their membranes. Because metal ions are vital for several life processes, and their action also inflicts damage on DNA and proteins, their proper distribution is vital and a slight alteration in their activity could cause severe disease. For example, abnormal iron uptake has been implicated in the most common hereditary disease hemochromatosis, as well as in neurological diseases such as Parkinson's disease, Friedreich's ataxia and Pica (Babcock *et al*., 1997; Andrews and Levy, 1998; Askwith and Kaplan, 1998). Apparently, any aberration in the cellular metal ion concentrations may cause a shortage of a vital metabolic element or inflict damage that may lead to cell death. Therefore, limited activity of a single metal ion transporter may cause growth arrest, and excess activity of the same transporter may be toxic, leading to cell death.

Recent studies on metal ion transporters painted a picture of coordinated action of uptake and secretion systems for achieving the proper homeostasis for individual tissues. In some of the cellular organelles and the plasma membrane, low and high affinity transporters act in concert to maintain the right balance of metal ion concentrations (Hediger, 1997; Eide, 1998; Radisky and Kaplan, 1999). Moreover, highly specific transport systems function along with a wide range of metal ion transporters to achieve the right concentration balance. The vacuolar system of eukaryotic cells is a major player in metal ion homeostasis not only because it provides several organelles for storage of metal ions but also by providing the proper amounts of transporters in the various cellular membranes through the secretory pathway. In addition, the organelles that evolved from bacterial endosymbiosis (mitochondria, chloroplasts and peroxisomes) serve as vital metal ion reservoirs and contribute to the overall metal ion homeostasis by utilizing their own transport and storage systems (Nelson, 1992; Supek *et al*., 1997). The field of metal ion transporters is in its infancy; therefore I will put forward some examples while neglecting others.

Compulsion and punishment

Metal ion homeostasis is governed by two evolutionary consequences. (i) Because redox reactions are fundamental life processes and transition metals are essential for the function of most proteins involved in redox reactions, living cells compete for metal ion resources. (ii) The development of several life processes involves toxic reagents that, when present in abnormal amounts, cause damage to the very function that they serve and to proteins and nucleic acids that are present in their proximity. The brain utilizes components such as NO, CO and metal ions that inflict damage during their normal physiological action (Supek *et al*., 1997). Moreover, the tendency of the brain to operate on the edge is exemplified by the utilization of glutamic acid as the major excitatory neurotransmitter. As a chemical, glutamic acid is not toxic and can be accumulated in the cytoplasm of most cells to relatively high levels. Following the adoption of glutamic acid as a neurotransmitter, specific systems evolved to render it highly toxic when present outside the brain cells. The same principle holds for the utilization of transition metals in several metabolic and neuronal functions.

Metal ion transporters provide an efficient tool for competition for the limited resources, and at the same time their regulation should provide solutions to the changing environment and the potential damage inflicted by abnormal concentrations. The answer to the challenging demand was given by providing the living cells with a wide variety of metal ion transporters acting in concert in the various organelles and cellular membranes. The different transporters can be grouped into those that are driven by the chemical energy of ATP and those that are driven by electrochemical gradients of protons and other ions. Some

of the systems are built up by couples of transporters, one of high affinity and low capacity and the other of low affinity and high capacity (see Eide, 1997).

The different metal ions may be grouped into redox-active ions such as Fe^{2+}, Cu^{2+}, Co^{2+} and to a lesser extent Mn^{2+}; and non-redox-active ions such as Ca^{2+} and Zn^{2+}. Zinc and calcium may be targeted to transcription factors and other enzymes involved in DNA metabolism, because the presence of redox-active metal ions in these places can lead to the promotion of radical reactions that result in nucleic acid damage. The redox-active ions normally function in enzymes that participate in redox reactions and the conversion of active oxygen-containing components. All of these processes require defined amounts of specific metal ions at the right position and in a timely fashion.

The mixed blessing of iron utilization

The electronic structure of the two redox states of iron, Fe^{2+} and Fe^{3+}, renders them the most versatile cofactors in biological redox reactions. Even though iron is a major component of our planet, its availability on the earth's surface is limited. The amounts of iron in the oceans limit the proliferation of phytoplankton and essentially limit the total mass of other organisms that are higher up in the food chain (Behrenfeld and Kolber, 1999). Consequently, both bacteria and eukaryotes have developed extremely efficient systems that can compete for the iron resource at subnanomolar concentrations. They produce and secrete siderophores which are iron chelators that can bind iron very tightly (down to 10^{-19} M). The loaded chelators can be either taken up or provide the iron to the plasma membrane high affinity transporters (Ardon *et al*., 1998; Atkinson *et al*., 1998; Fisher *et al*., 1998; Lesuisse *et al*., 1998). The global iron limitation was created by the generation of an oxidative atmosphere by photosynthetic cyanobacteria (Lewin, 1976). This in turn oxidized most of the surface iron into Fe^{3+} that is insoluble at neutral and high pH and is not available to the organisms. On the other hand, Fe^{3+} can be tightly bound to several compounds, and thus most of the siderophores and the cellular iron-binding proteins such as transferrin and ferritin bind and store iron in its oxidized form (Kaplan and O'Halloran, 1996). Reduction of the bound Fe^{3+} to Fe^{2+} and/or lowering the pH are utilized by the different systems for the release of the bound iron and for making it available for transport and the required enzymatic and metabolic processes (Shatwell *et al*., 1996).

Mammals usually have an abundant supply of iron in their food, and their main concern is to regulate the iron supply according to the specific demands of the different tissues and specialized cells. Because of the potential damage inflicted by the presence of iron in wrong compartments or at excessive concentrations, a very elaborate system of uptake, storage and release was developed (Hediger, 1997; Andrews *et al*., 1999). Initial studies with isolated mammalian cells indicated that iron transport is mediated predominantly by the transferrin system. This pathway involves the adsorption of iron onto transferrin, followed by the binding of the Fe–transferrin to its receptor, internalization of transferrin-bound receptors into endosomes, thereby releasing the bound iron by the low pH generated by V-ATPase, and finally iron transport across the endosomal membrane into the cytoplasm (Supek *et al*., 1997; Gruenheid *et al*., 1999; Nelson and Harvey, 1999). A defect in the transferrin pathway results in iron deficiency, suggesting the predominance of this pathway. Apparently, mammalian cells experience an iron stress that can be overcome by the tight binding of iron onto transferrin at neutral pH and its release at low pH. This would provide an iron uptake system that can compete with the natural iron-chelating materials that are present outside the cell. Since the affinity of most iron chelators decreases at low pH, a similar mechanism of iron uptake, operating through the endocytic pathway, may exist in most eukaryotic cells.

A closer look at the overall iron intake into mammalian cells revealed that the receptor-mediated iron uptake is accompanied by transporter-mediated iron uptake (Jordan and Kaplan, 1994). It was also apparent that even the transferrin-mediated iron transport should be accompanied by a transporter that would facilitate the iron transport across the endosomal membrane (Supek *et al*., 1997). Recent studies indicate a central role for these transporters in iron homeostasis (Fleming *et al*., 1997; Gunshin *et al*., 1997; Andrew *et al*., 1999). The discovery that the yeast homolog Smf1p of the mammalian Nramp (natural resistance-associated macrophage protein) is a metal ion transporter paved the way for this advancement (Supeck *et al*., 1996). Subsequently, it was demonstrated that the two mammalian homologs of Smf1p are broad-range metal ion transporters and may play a crucial role in iron absorption from the duodenum as well as transport of the low pH released iron in the endosomes. Figure 1 depicts a balanced view of the function of key elements in transporters and receptor-mediated iron uptake. Since oxidized iron is more abundant outside the mammalian cells and Nramp2 (DCT1 or DMT1) transports iron only in its reduced form, a copper-mediated Fe^{3+} reduction to Fe^{2+} is the first step in iron transport (Askwith and Kaplan, 1998). The mechanism of Fe^{2+} secretion from the cell is not clear, but in the blood stream another copper protein, ceruloplasmin, oxidizes Fe^{2+} to Fe^{3+} and makes it amenable to bind apotransferrin. Subsequently, transferrin binds to the transferrin receptor and is internalized by endocytosis to the endosomes; the iron is released by the low pH generated by V-ATPase and is transported into the cytoplasm by Nramp2 (Supek *et al*., 1997; Fleming *et al*., 1998; Gruenheid, *et al*., 1999). The amount of free iron in the cells is controlled by the amount of its uptake and the amounts of iron stored in ferritin. These processes are regulated inversely by the iron regulatory protein (IRP) which is a non-heme-ion protein with enzymatic activity of aconitase at high iron levels and which binds to an iron-responsive element (IRE) after losing the iron at low iron concentrations (Gehring *et al*., 1999). The importance of iron storage in the mitochondria was elucidated by the study of Yfh1p, which is a homolog of the human frataxin (Babcock *et al*., 1997). Decreased amounts of the human protein cause Friedreich's ataxia. Several recent reviews cover the subject of iron uptake and storage in different compartments of eukaryotic cells (Hediger, 1997; Andrews and Levy, 1998; Askwith and Kaplan, 1998; Eide, 1998;

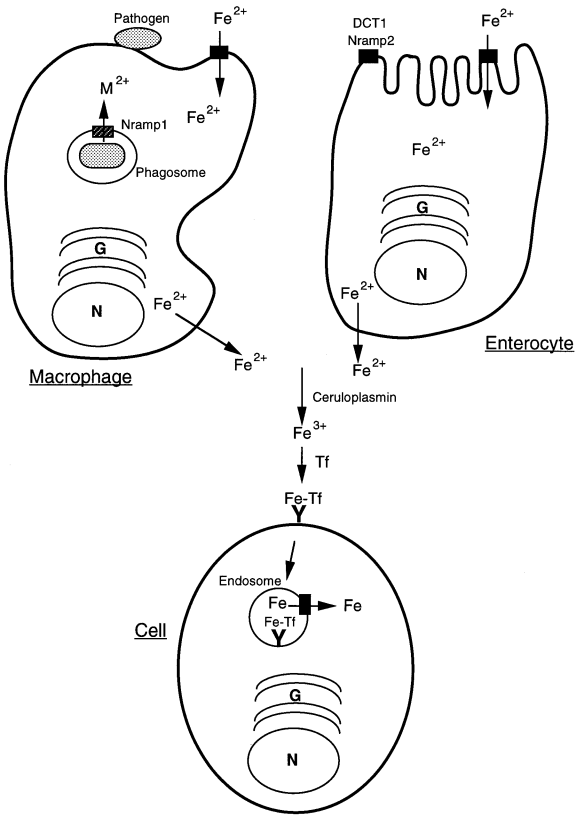

Fig. 1. Schematic representation of key elements in iron transport and homeostasis. The different tissues are represented by a duodenal cell (enterocyte), a blood cell (macrophage) and a parenchymal cell such as an hepatocyte (cell). It is proposed that DCT1 (Nramp2) is the main port of iron entry in the duodenum. The reduced iron is provided directly from the food (meat) or it is enzymatically reduced on site. Iron is competing for transport with other metal ions. The mechanism of iron secretion from the cell is not specified. In the blood stream, the iron is oxidized by ceruloplasmin, binds to transferrin and transferrin receptors of different cells, is taken up by endocytosis, liberated in the endosomes by the acidic pH and transported to the cytoplasm by Nramp2 or Nramp1 in specific cells such as macrophages. N, nucleus; G, Golgi; M, metal ion; Tf, transferrin; Y, transferrin receptor.

Andrews *et al*., 1999; Radisky and Kaplan, 1999), but the subject is far from exhausted.

Studies in yeast and their implication for other eukaryotes

Genetic screens have identified several yeast genes that encode metal ion transporters or are involved in metal ion homeostasis. Some of these genes are specific for fungi; others belong to gene families that expand from bacteria to human. Some yeast and human homologous proteins were found to be related to copper and iron transport (Andrews and Levy, 1998; Eide, 1998; Radisky and Kaplan, 1999), but the most ubiquitous proteins discovered in that way are the metal ion transporters encoded by the *SMF* gene family (Supek *et al*., 1996, 1997). *SMF1* was originally cloned as a high copy number suppressor of a temperature-sensitive *mif1-1* mutant (West *et al*.,1992). *MIF1* (*MAS1*) and *MAS2* (*MIF2*) encode the processing-enhancing protein and the matrix-processing peptidase, respectively. The two proteins function as a heterodimer to form the active holoenzyme of mitochondrial processing peptidase, which is vital for cell growth (Pollock *et al*., 1988; Witte *et al*., 1988; Yang *et al*., 1988). The activity of the purified peptidase is inhibited by chelators such as EDTA or orthophenantroline and is stimulated by Mn^{2+}, Zn^{2+} or Co^{2+} (Hawlitschek *et al*., 1988). The temperature-sensitive *mif1-1* mutant may result from reduced stability of the processing peptidase under limited manganese concentrations in the medium (Supek *et al*., 1996). Similarly, Cdc1p may be a Mn^{2+}-dependent cell division cycle protein that is also vital for cell growth (Paidhungat and Garrett, 1998a,b). A *csp2* mutant that is sensitive to the presence of EGTA in the medium was generated by ethyl methanesulfonate (EMS) treatment of yeast cells (Supek *et al*., 1996). It was demonstrated that the Csp^{-} phenotype of the *csp2* mutant is caused by a missense mutation in the *CDC1* gene, in which Gly149 was substituted by arginine. Therefore, the *cdc1-1* (*csp2*) mutant exhibited very similar characteristics to the *mas1* (or *mif1-1*) mutant, with the exception of their growth-inhibiting conditions (which are EGTA and 37°C, respectively). In both cases, the block can be relieved by supplementing the media with Mn^{2+} or overexpressing SMF1 that transports Mn^{2+} from the medium and elevates its concentration in the cytoplasm (Supek *et al*., 1996; Liu *et al*., 1997). Further studies indicated that SMF1 is a general metal ion transporter and can transport not only Mn^{2+}, Zn^{2+} and Cu^{2+} (Supek *et al*., 1996), but also Fe^{2+}, Cd^{2+}, Ni^{2+} and Co^{2+} (Liu *et al*., 1997; Supek *et al*., 1997; X.Z.Chen, J.B.Peng, A.Cohen, H.Nelson, N.Nelson and M.A.Hediger, submitted). Yeast cells contain two additional genes of this family, *SMF2* and *SMF3*, and indirect evidence indicates that their products are also broad-range metal ion transporters but exhibit different specificity from that of SMF1 (A.Cohen, H.Nelson and N.Nelson, unpublished).

A mutation in the *malvolio* (*mvl*) gene affects taste behavior in *Drosophila*. The gene is expressed in mature neurons in the central and peripheral nervous system as well as in macrophages (Rodrigues *et al*., 1995). It was shown that the electrophysiological responses of the peripheral neurons to taste stimuli are normal in these flies. This suggests that the abnormal taste behavior of the mutant resulted from a defect in information processing rather than in the reception of the stimulus (Rodrigues *et al*., 1995). Since the amino acid sequence of MVL is 65% identical to that of the mammalian Nramp (Vidal *et al*., 1993, 1995; Cellier *et al*., 1995), it is likely that both of them have a similar function. The discovery of a yeast protein (Smf1p) that is ~30% identical in its amino acid sequence to Nramp and MVL raised the possibility that the mammalian and *Drosophila* proteins also have a similar function (Supek *et al*., 1966). According to this hypothesis, metal ion homeostasis is impaired in the *MVL* mutant, resulting in a loss of taste perception for sugars. To test this hypothesis, *MVL* mutant flies were allowed to develop from eggs to adulthood on a medium containing elevated concentrations of metals. Mutant flies that were reared in the presence of 10 mM $MnCl_2$ or $FeCl_2$ developed into adults with recovered taste behavior (Orgad *et al*., 1998). Furthermore, exposure of adult mutant flies to these ions in the testing plate for only 2 h was sufficient to restore normal taste behavior. The suppression of defective taste behavior suggests that the MVL protein functions as an Mn^{2+} and Fe^{2+} transporter and that Mn^{2+} and/or Fe^{2+}

are involved in the signal transduction of taste perception in *Drosophila* adults (Orgad *et al*., 1998). The role of Mn^{2+} and Fe^{2+} in neurotransmission and taste perception is not clear. Their possible involvement in neural development was ruled out since Mn^{2+} and Fe^{2+} suppressed the mutant phenotype even when supplied to flies only at the adult stage, when the nervous system had already been developed. Alterations in metal ion homeostasis are known to result in several neurological diseases, and the *Drosophila* mutants may provide a useful genetic tool to study their molecular mechanism.

Most of the plasma membrane transporters utilize electrochemical gradients as the driving force for their transport activity. Several other transporters utilize ATP for transporting substances across the membrane. Most of them belong to the superfamily of P-type ATPases that are integral membrane proteins, having a similar structure and mechanism of action to those of Na^+/K^+-ATPases and Ca^{2+}-ATPases (Maeda *et al*., 1998). The *CCC2* gene in *Saccharomyces cerevisiae* encodes such an ATPase (Ccc2p) that exports copper into the lumen of post-Golgi vesicles (Fu *et al*., 1995; Yuan *et al*., 1997). Like *SMF1*, this gene was cloned by a complementation of the seemingly unrelated mutation *csg1*, which exhibits Ca^{2+} sensitivity. Several of the ATP-dependent pumps function in providing electrochemical energy to secondary processes as well as detoxification of the cytoplasm by exporting a wide variety of substances (Petris *et al*., 1996). Originally Ccc2p may have functioned in transporting excess copper from the cytoplasm. The bacterial homologs of Ccc2p may fulfill this function (Phung *et al*., 1994). In in eukaryotic cells, the main function of this transporter apparently is to supply copper to an organelle of the post-Golgi network, where assembly of a complex that contains a copper-dependent oxidoreductase and an iron permease (Fet3p in yeast) takes place (Culotta *et al*., 1999). Consequently, mutations in Ccc2p or its mammalian homolog result in iron deficiency.

The vacuolar system of eukaryotic cells serves as a major storage site for metal ions and plays a crucial role in the homeostasis of calcium and other metal ions (Nelson and Harvey, 1999). The vacuolar H^+-ATPase (V-ATPase) provides the protonmotive force for several of the vacuolar transport systems. Some other transport processes are driven by primary pumps that are P-type ATPases. Manganese homeostasis is modulated by Smf1p and Smf2p, which are plasma membrane residents and by the P-type ATPase Pmr1p, which is situated in the vacuolar system (Antebi and Fink, 1992). Pmr1p was discovered as a potential Ca^{2+}-ATPase required for normal Golgi function (Rudolph *et al*., 1989; Antebi and Fink, 1992; Okorokov *et al*., 1993). Later, it was discovered that Pmr1p supplies the secretory pathway not only with Ca^{2+} but also with Mn^{2+}, which is required for glycosylation and protein sorting as well as degradation in the endoplasmic reticulum (Durr *et al*., 1998; Okorokov and Lehle, 1998). It is remarkable that until recently manganese was not considered to be an essential element for yeast growth, and only by discovering that mutations in Cdc1p and Mas1p can be complemented by the addition of Mn^{2+} did it become apparent that this metal ion is vital (Supek *et al*., 1996; Paidhungat and Garrett, 1998a,b). Manganese functions as a cofactor of some glycosylation enzymes (Durr *et al*., 1998). Even though, individually, not one of them is essential for growth, it is possible that collectively they are vital, and inhibiting their activity through lack of manganese results in growth arrest. The analogy between the uptake systems and site of activity between iron and manganese is quite apparent. Both systems utilize plasma membrane transporters that are probably driven by electrochemical gradients for the metal ion supply and P-type ATPases that function to provide the metal ions to the lumen of the vacuolar system, facilitating reactions that require the specific metal ion.

The mechanism of metal ion transport by eukaryotic cells is largely obscure. Most of the information came from electrophysiological studies on DCT1 (Nramp2) and Smf1p that were expressed in *Xenopus* oocytes (Gunshin *et al*., 1997). Figure 2 depicts experimental and kinetic elements that may help in understanding the complexity of this transport system. Yeast cells grow at a relatively acidic pH and their plasma membrane is energized primarily by the P-type proton pump Pma1p (Nelson, 1992). Consequently, most of their transport systems are driven by the protonmotive force and several of the transported substances are taken up by co-transport with protons. Mammalian cells grow at neutral pH and most of them utilize a sodium electrochemical gradient generated by the Na^+/K^+-ATPase for driving their uptake systems. Usually sodium is co-transported across the membrane with the various substances (Wright *et al*., 1994). It was therefore quite surprising to discover that some of the plasma membrane mammalian transporters that operate at neutral pH co-transport protons with their substrates (Mackenzie *et al*., 1996; Steel *et al*., 1997; Chen *et al*., 1999a). It was demonstrated that DCT1 co-transports Fe^{2+} together with H^+ with a stoichiometry of 1:1 (Gunshin *et al*., 1997). Replacing Na^+ by choline and Cl^- by NO_3^- or SCN^- had no effect on the Fe^{2+} transport (Gunshin *et al*., 1997). At physiological membrane potentials of –90 to –30 mV, the apparent affinity constant for H^+ was ~1 μM, suggesting that the membrane potential component of the protonmotive force provides most of the driving force for Fe^{2+} transport. DCT1 expressed in *Xenopus* oocytes induces a proton leak at low pH and, under certain conditions, may operate as an H^+ uniporter (Gunshin *et al*., 1997). The yeast Smf1p, expressed in *Xenopus* oocytes, exhibited quite similar properties to DCT1 (X.Z.Chen, J.B.Peng, A.Cohen, H.Nelson, N.Nelson and M.A.Hediger, submitted). The most striking difference was observed for the uncoupled leak, which was specific for Na^+ and not H^+. The physiological significance of this phenomenon is not clear, but similar uncoupled leaks were demonstrated for the Na^+-Cl^--dependent GABA transporter GAT1 and the Na^+/glucose transporters SGLT1 and SGLT2 (Loo *et al*., 1993; Mager *et al*., 1993, 1996). The influences of substrates and inhibitors on the leak currents suggest that they are part of the transport mechanism (Wright *et al*., 1994; Nelson, 1998).

Since it is unlikely that the transport of a divalent cation such as Fe^{2+} will be driven by additional cation H^+, we further studied the influence of anions on the currents generated by expressed DCT1 (Nramp2) in *Xenopus* oocytes (A.Sacher, A.Argaman and N.Nelson, unpublished). A two-electrode recording of currents induced by electrogenic transporters with or without

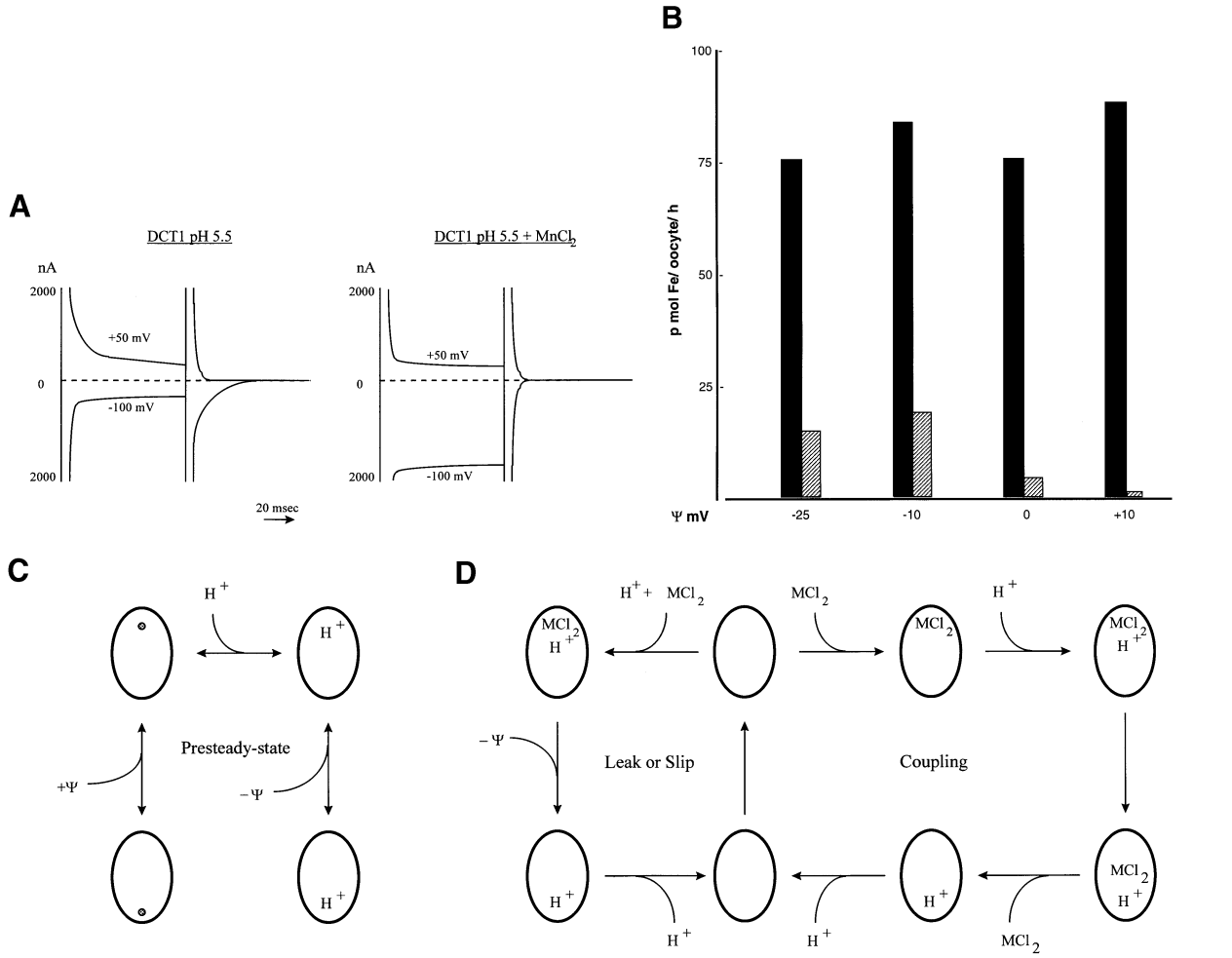

Fig. 2. Schematic representation of the minimal steps involved in the pre-steady-state currents and metal ion transport of the SMF-like transporters expressed in *Xenopus* oocytes. (**A**) Schematic presentation of two-electrode voltage-clamp analysis of currents generated by DCT1 expressed in *Xenopus* oocytes at +50 or –100 mV. The imposed voltage lasted for 100 ms and an additional 100 ms were recorded upon return to the holding potential of –50 mV. In the absence of metal ions, a pre-steady-state current is observed only at a positive imposed potential. Upon return to the holding potential, a discharge of the pre-steady-state current is recorded. Addition of $MnCl_2$ causes a relatively small outward current at the positive potential and a large current at the negative imposed potential. The large current at the negative potential is composed of transport plus large leak or slip. (**B**) $^{55}Fe^{2+}$ uptake at different holding potentials in the presence (solid bars) and absence (hatched bars) of chloride ions in the medium. The chloride was substituted by 100 mM gluconate. A concentration of ~2 mM Cl^- is present in the gluconate solution. (**C** and **D**) Schematic description of the kinetic coupling and the sequential binding of protons (H^+), chloride (Cl^-) and metal ion (M) as well as the influence of membrane potential (ψ) on the different steps in the mammalian metal ion transporter DCT1 (Nramp2). In the presence of chloride and the absence of metal ions and only under positive potential (C), the proton-binding site (dot) is exposed. The proton can bind, move to the internal face of the membrane and generate pre-steady-state current. Upon return to the holding potential of –50 mV, the process is reversed. Under a physiological negative potential (D), the metal ions binding open up the proton-binding site and, following translocation inside the oocyte, they are released and a steady-state current is generated. In the presence of metal ions and negative potential, a large leak current (slip) of protons is generated (left).

their substrates reveals not only the steady-state but also pre-steady-state currents (Wright *et al*., 1994; Loo *et al*., 1998). The steady-state current is the result of net charges transported across the membrane with time and is proportional to the number of active transporters and to their turnover under the given driving force. The pre-steady-state reflects the exposure or temporary transport of charges on or across the oocyte membrane under an applied potential. As shown in Figure 2A, upon return to the holding potential, the pre-steady-state current will be discharged as a mirror image of the induced one (Loo *et al*., 1998). The number of expressed transporters per oocyte can be calculated from the integrals of the transient currents (Wright *et al*., 1994), and these numbers could be verified by counting the particles obtained by freeze-fracture electron microscopy (Eskandari *et al*., 1998). Oocyte-expressed DCT1 exhibits large pre-steady-state currents but only at positive potentials (Gunshin *et al*., 1997). The appearance of pre-steady-state currents only at positive applied potentials is a critical aspect in the kinetic puzzle. As shown in Figure 2C, we propose that positive potentials expose the proton-binding site on the outer surface of the transporter (depicted as a dot that moves from the inside out). The proton binds at low pH with an affinity constant of ~1 μM and the binding free energy is sufficiently larger than the inside positive potential such that the proton can move to the internal face of the transporter. This movement is expressed as a slow (half-life ~20 ms) inward positive current. Going back to the holding potential (–50 mV), the pre-steady-state relaxes as an outward positive current with similar kinetics (Figure 2A, left). Addition of metal ion results in

the disappearance of the pre-steady-state current, either by preventing the inward proton movements (see below) or under competent transport conditions by releasing the proton and the metal ion from the membrane to the cytoplasm (Figure 2A and D, right). Both actions would result in the disappearance of the pre-steady-state current. Very recently, we observed that substituting Cl^- in the transport solution with gluconate or isethionate results in the appearance of large pre-steady-state currents not only at positive but also at negative applied potential. Moreover, substitution of chloride anions by gluconate drastically reduced the $^{55}Fe^{2+}$ uptake into the oocytes and rendered it sensitive to membrane potential (Figure 2B). In the presence of chloride, the $^{55}Fe^{2+}$ uptake was insensitive to imposed membrane potentials from –75 to +50 mV (Figure 2B, and unpublished observations). Therefore, the metal ion transport is dependent on the presence of Cl^- or other small anions such as NO_3^- or SCN^- but not SO_4^{2-}. We propose in Figure 2D that the metal ion is co-transported with Cl^-, and the steady-state current results from the transport of positive charges of H^+. The kinetic scheme depicted in Figure 2 explains some but not all of the observations mentioned above. The physiological virtue of the leak or slip currents in DCT1 and Smf1p can be explained in terms of prevention of excess metal ion uptake under stress. In the course of our studies on other transport systems, we came to realize that there is no biological process without a slip and that understanding transport processes requires unraveling of the mechanism of their slippage. The kinetic experiments with DCT1 and Smf1p expressed in *Xenopus* oocytes, together with the available yeast mutants, provide a solid foundation for a detailed study of the mechanism of metal ion transport across membranes.

Metal ion transport in mammalian cells and human diseases

Metal ion homeostasis and host defense against bacterial infection

There has been an upsurge of tuberculosis in many parts of the world in the past decade. The most catastrophic phenomenon is the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*. These organisms have caused epidemic outbreaks in healthcare institutes in the USA and some European countries. Due to the development of antibiotic resistance, there is a quest for other novel modalities of therapy. The cloning of *Nramp1* identified a gene responsible for the resistance or sensitivity of mice to mycobacteria and defined a novel target for therapeutic agents (Vidal *et al*., 1993). *Nramp1* is identical to the *Ity* and the *Lsh* genes conferring resistance to infection by *Salmonella typhimurium* and *Leishmania donovani*, respectively (Plant *et al*., 1982). Although the cloning of *Nramp* identified the gene responsible for resistance of mice to mycobacteria, its function was unknown. The identification of Smf1p as the yeast Nramp homolog, and a possibly similar function for those two proteins suggested that the mammalian protein functions as a scavenger of metal ions from the bacteria-containing phagosomes (Supek *et al*., 1996). Figure 3 depicts a proposed model for the role of Nramp1 in macrophage defense against microbial invasion. Following the phagocytosis of a parasite into the phagosome, the macrophage produces reactive oxygen and/or nitrogen intermediates that are toxic for the internalized bacteria (Segal and Abo, 1993). The survival of the pathogen during the burst of macrophage respiratory activity is mediated by microbial enzymes, most of which contain metal ions in their active centers (Chan *et al*. 1992). It was proposed that Nramp1, like its yeast homolog, transports metal ions from the phagosomal lumen into the cytoplasm (Supek *et al*., 1996, 1997). Thus the metal ion depletion of the phagosomal lumen becomes a rate-limiting step in metalloenzyme production by the engulfed bacteria. This will restrict mycobacterial ability to produce active enzymes such as superoxide dismutase (SOD) and prevent the propagation of the ingested microorganisms. Conversely, an increased concentration of metal ions in the phagosome caused by a defective Nramp transporter (Bcg^s) may promote the growth of the mycobacteria and render the organism sensitive to the pathogen. The discovery of *Nramp* homologous genes in several bacteria suggests that the pathogens use the same strategy in competition for the limited amounts of metal ions inside the phagosome. Several

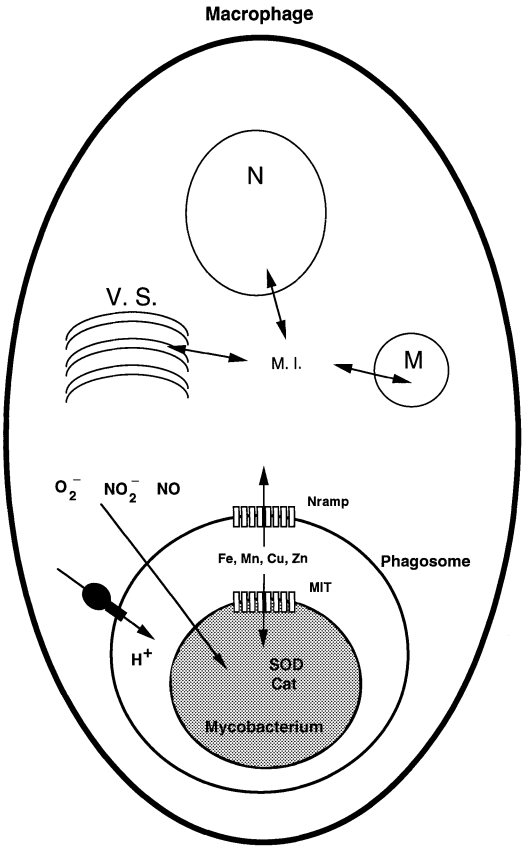

Fig. 3. The function of Nramp and its bacterial homolog MIT in macrophage–pathogen interaction. The infecting bacterium is taken into the macrophage phagosome where it is challenged by reactive oxygen components. The bacterium protects itself by producing metalloenzymes such as catalase and superoxide dismutase that can neutralize the toxic agents. For that it needs a large supply of metal ions that are taken up from the macrophage into the bacterium by its metal ion transporter MIT. The macrophage defense is based on the deprivation of the bacterium of the required metal ions by the activity of the metal ion transporter Nramp1. N, nucleus; V.S., vacuolar system; M, mitochondria; M.I., metal ions; SOD, superoxide dismutase; Cat, catalase.

recent studies support this proposal (Agranoff and Krishna, 1998; Fleming and Andrews, 1998). Studies with yeast mutants may provide an amenable system for discovering new drugs targeted specifically against the bacterial transporters. It was demonstrated that the double disruptant mutant in which *SMF1* and *SMF2* were inactivated fail to grow at pH 8 (Pinner *et al*., 1997). The restricted growth of this mutant could be complemented by the expression of *Nramp2*. We generated a triple disruptant mutant in which all three *SMF* genes were inactivated (A.Cohen, H.Nelson, J.Voss and N.Nelson, unpublished). This mutant is unable to grow at pH 7.5 and could be complemented by expression of the metal ion transporter MIT from *Mycobacterium leprae*. A drug against mycobacteria can be developed by looking for components that inhibit MIT-dependent yeast growth and have no effect on *Nramp1*-dependent yeast growth.

Hemochromatosis

Hemochromatosis is the most prevalent hereditary disease, with an estimated carrier frequency of ~10% (Edwards *et al*., 1988). The progression of the disease is a result of excess iron accumulation that is deposited in a variety of organs. The excess iron causes progressive organ failure resulting in various illnesses such as diabetes, hepatoma, cirrhosis, cardiomyopathy and arthritis (Bothwell *et al*., 1995). Severe effects of the disease usually appear in old age after decades of progressive iron loading. Therefore, the disease did not capture the front pages of newspapers. A gene (*HFE*) encoding MHC class I-like protein, in which two missense mutations were found in 85% of patients' chromosomes, was identified by positional cloning (Feder *et al*., 1996). The majority of the patients were homozygous for a C282Y substitution in the HFE protein. This mutation eliminates the ability of HFE to associate with β_2-microglobulin and prevented cell surface expression (Parkkila *et al*., 1997; Feder *et al*., 1998; Roy *et al*., 1999). How can an MHC-like protein, in association with β_2-microglobulin, control iron homeostasis and cause hemochromatosis when mutated? The best candidate was the transferrin receptor that was found to be associated with HFE (Waheed *et al*., 1999). The association was demonstrated as taking place in crypt enterocytes of the duodenum, where it has a unique intracellular localization. However, the major site of iron uptake is in the villus, where the receptor has little or no function in iron transport into the cells. The discovery of Nramp2 (DCT1 or DMT1) as an iron transporter and its localization in villus cells suggest a major function for this metal ion transporter in iron uptake from the duodenum to the cells (Gunshin *et al*., 1997; Andrews *et al*., 1999; Garrick *et al*., 1999). Recently, a reciprocal regulation of *HFE* and *Nramp2* gene expression by iron in human intestinal cells was reported (Han *et al*., 1999). While *HFE* mRNA and protein were increased by increasing cellular iron concentrations, the level of *Nramp2* mRNA decreased. Thus, the modulation of *Nramp2* is in line with cellular needs. It is proposed that iron absorption from the duodenum to the enterocyte cells is modulated by the amounts of Nramp2 present on the plasma membrane (Fleming *et al*., 1999). The HFE functions in these cells directly or indirectly as a negative regulator of Nramp2 trafficking to the plasma membrane. Large amounts of HFE would hold the Nramp2 in the vacuolar system (Golgi or endosomes), and decreasing iron concentrations in the external milieu would cause the release of Nramp2 and its deposition on the plasma membrane. Furthermore, mutated HFE results in failure of holding Nramp2 in the vacuolar system, followed by excess iron uptake that eventually causes symptoms of hemochromatosis. A similar mechanism was reported for Smf1p in yeast cells (Liu *et al*., 1997; Liu and Culotta, 1999). The Bsd2p was shown to hold the Smf1p in the vacuolar system, and in its absence much more of the transporter was deposited on the plasma membrane. This proposed mechanism is in line with several other regulatory systems of transport processes including the insulin-induced regulation of glucose transporter trafficking (Wright *et al*., 1994) and the regulation of monocarboxylate transporters by OX-47 (Juel and Halestrap, 1999). Very recently, a locus on the long arm of chromosome 1 was identified as the location bearing the gene responsible for juvenile hemochromatosis (Roetto *et al*., 1999). Affected juveniles did not show linkage to chromosome 6p and do not have mutations in the *HFE* gene. It will be very interesting to determine whether the new gene influences the distribution and/or activity of the metal ion transporter DCT1 or the receptor-mediated iron uptake.

Metal ion homeostasis and neurological diseases

Transport of metal ions from the blood to the brain involves the crossing of the blood–brain barrier. Studies of iron transport in the brain showed that the blood–brain barrier permeability of Fe–transferrin is similar to that of albumin (Morris *et al*., 1992). The experiments suggested that uptake of iron into the brain involves the transport of iron from iron-loaded blood transferrin to a brain-derived transferrin for extracellular iron transport within the brain (Morris *et al*., 1992). Subsequently, the iron is taken up via transferrin receptors into the brain cells. High peripheral iron concentrations in hemochromatosis patients result in elevated iron levels in the brain (van Gelder *et al*., 1998). Perturbation in iron homeostasis was observed by chronic treatment of rats with chlorpromazine, a medication for schizophrenic patients (Ben-Shachar *et al*., 1994). This observation suggests that some of the side effects caused by psychotic drugs may be due to perturbation of metal ion homeostasis. Manganese is also readily taken up into the central nervous system, most likely as a free ion (Murphy *et al*., 1991; Rabin *et al*., 1993). However, the transport is affected by plasma proteins such as albumin and transferrin that bind Mn^{2+}. Except for its function in metalloproteins, there is no indication of specific neuronal modulation by manganese. However, the recent observation that Mn^{2+} and Fe^{2+} affect the taste behavior of *Drosophila* suggests that metal ions may function in novel signal transduction pathways in the brain (Orgad *et al*., 1998). Recent studies demonstrated that zinc can be considered as a neurotransmitter because it is accumulated in presynaptic vesicles of excitatory neurons, is released with synaptic activity, interacts with some ionotropic receptors and is taken back by a specific transporter (Assaf and Chung, 1984; Howell *et al*., 1984; Peters *et al*., 1987; Westbrook and Mayer, 1987; Seguela *et al*., 1996; Sensi *et al*., 1997; McMahon and Cousins, 1998). Zinc can interact strongly with a variety of ligands including sulfur in cysteine, nitrogen in histidine and

oxygen in acidic amino acids. Therefore, it is likely to be bound to serum proteins, and it may cross the blood–brain barrier as a ligand of amino acids or other components that bind zinc. In light of the recent observation of stress-related breaks in the blood–brain barrier (Kaufer *et al*., 1998), the mechanism of metal ion accumulation in the brain should be re-evaluated.

Because metal ions are critical for several metabolic processes and are poisonous at moderate or high concentrations, it is not surprising that growing numbers of neurological diseases are connected to metal ion homeostasis. The interplay of specific and general metal ion transporters and the connection between copper and iron transport cause multifarious alterations in metal ion homeostasis in each genetic aberration (Radisky and Kaplan, 1999). In addition, individual physiological adaptations can take place during the development of persons bearing an identical mutation in a metal ion transporter. If the mutation causes a mild alteration in the transport activity but does not cause juvenile lethality, each individual may adapt differently to the alteration and consequently experience a variety of symptoms, if any. One of the best examples of such an adaptation is the neurological disease Pica, which is probably a result of low iron concentrations in certain parts of the brain (Moore and Sears, 1994). Individuals who are presumably affected by the same iron shortage manifest different psychological behaviors that can be grouped collectively as Pica. This phenomenon is also common in knockout mice that may develop somewhat different phenotypes as a result of identical genetic alteration. The symptoms of Pica include a compulsive ingestion of non-food substances including dirt, ice, soap, ashes, chalk, paint and wooden sticks (Moore and Sears, 1994). The onset of the disease may be connected to heredity, environmental hazards and physiological conditions, and may be manifested at different ages, or during pregnancy, illness and stress. It is now well accepted that iron deficiency is the cause of Pica, but the etiology of the disease is poorly understood. The recent discovery that manganese and iron may be involved in taste perception in *Drosophila* (Orgad *et al*., 1998) may provide a model system for the study of the neurological consequences of variations in metal ion concentrations.

Copper homeostasis is critical for mammalian cells not only because it is necessary for several redox enzymes and to maintain iron homeostasis but also because of its toxicity. Copper is present outside the cells as the oxidized Cu^{2+} form and may be transported in this form by the metal ion transporters DCT1 in mammals and Smf3p in yeast (Hediger, 1997; A.Cohen and N.Nelson, unpublished). Two yeast genes encoding membrane proteins (Ctr1p and Ctr3p) that are involved in the uptake of reduced copper Cu^{+} were identified and studied in detail (Dancis *et al*., 1994a,b). Although their synthesis and degradation are highly regulated by the external copper concentrations, the mechanism of copper transport into the yeast cells is largely unknown (Ooi *et al*., 1996; Labbe *et al*., 1997). cDNAs encoding homologous proteins in *Arabidopsis* and humans were cloned and shown to complement the yeast null mutant *ctr1-3* (Kampfenkel *et al*., 1995; Zhou and Gitschier, 1997). Much more is known about the structure and function of the vacuolar P-type ATPase copper transporter (Ccc2p) and its human homologs that are responsible for Wilson's and Menkes diseases (Fu *et al*., 1995; Hung *et al*., 1997; Yuan *et al*., 1997). These diseases are inherited disorders of copper metabolism resulting from the absence or dysfunction of homologous copper-transporting ATPases that reside in the *trans*-Golgi network of the cell (Schaefer and Gitlin, 1999). The prevalence of Wilson's disease in which copper accumulates in the liver, kidney, brain and cornea is ~30 per million. The homozygotic gene carriers eventually develop hepatitis, cirrhosis, amino aciduria and neurological deterioration, especially in the basal ganglia of the brain. The human gene encoding the copper-ATPase responsible for the disease was cloned and homozygous mutations were identified in patients (Bull *et al*., 1993; Petrukhin *et al*., 1993). There are splice variants of the gene including a recently discovered night-specific copper-transporting ATPase that exhibits a diurnal expression rhythm in the pineal gland and retina (Borjigin *et al*., 1999). Menkes disease is a fatal, X-linked, copper deficiency disorder that results from defective copper efflux from intestinal cells (Schaefer and Gitlin, 1999). The human gene encoding the copper-ATPase responsible for the disease was cloned, and qualitative or quantitative abnormalities in its mRNA were identified in patients (Vulpe *et al*., 1993). Menkes and Wilson's copper-ATPases function in the same manner within the cell and they were spotted at the same location in the secretory pathway. Moreover, expressed normal cDNAs of the respective genes corrected the copper transport defect of Menkes patient fibroblasts (La Fontaine *et al*., 1998). Therefore, the differences in the clinical expression of the two diseases can be a result of the unique tissue-specific expression of each protein and their specific function in copper detoxification by accumulation in the vacuolar system (Wilson's) and copper transport from intestinal cells to the blood (Menkes). The observation that in the presence of elevated copper concentrations there was a rapid trafficking of Menkes protein from the Golgi to the plasma membrane suggests that the cellular biology of the two copper-ATPases may be different (Petris *et al*., 1996). Thus, both proteins may function in copper homeostasis by similar but not identical mechanisms. They may supply the vacuolar system with enough copper to sustain the necessary iron uptake system and at the same time to protect the cells from excess copper either by accumulation in the vacuolar system or by secretion from the cell.

Hemochromatosis, Pica, Wilson's and Menkes diseases are only a few proven examples where etiology involves metal ion homeostasis. The onset of many other hereditary and environmental diseases, including Alzheimer's and Parkinson's, may eventually be connected with metal ions. Early detection and simple treatments with metal supplements or chelating agents may prevent the damage inflicted by the imbalances in metal ion homeostasis.

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References

Agranoff,D.D. and Krishna,S. (1998) Metal ion homeostasis and intracellular parasitism. *Mol. Microbiol*., **28**, 403–412.

Andrews,N.C. and Levy,J.E. (1998) Iron is hot: an update on the pathophysiology of hemochromatosis. *Blood*, **92**, 1845–1851.
Andrews,N.C., Fleming,M.D. and Levy,J.E. (1999) Molecular insights into mechanisms of iron transport. *Curr. Opin. Hematol*., **6**, 61–64.
Antebi,A. and Fink,G.R. (1992) The yeast Ca (2+)-ATPase homologue, PMR1, is required for normal Golgi function and localizes in a novel Golgi-like distribution. *Mol. Biol. Cell*, **3**, 633–654.
Ardon,O., Nudelman,R., Caris,C., Libman,J., Shanzer,A., Chen,Y. and Hadar,Y. (1998) Iron uptake in *Ustilago maydis*: tracking the iron path. *J. Bacteriol.*, **180**, 2021–2026.
Askwith,C. and Kaplan,J. (1998) Iron and copper transport in yeast and its relevance to human disease. *Trends Biochem. Sci.*, **23**, 135–138.
Assaf,S.Y. and Chung,S.H. (1984) Release of endogenous Zn^{2+} from brain tissue during activity. *Nature*, **308**, 734–736.
Atkinson,R.A., Salah El Din,A.L., Kieffer,B., Lefevre,J.F. and Abdallah,M.A. (1998) Bacterial iron transport: ^{1}H NMR determination of the three-dimensional structure of the gallium complex of pyoverdin G4R, the peptidic siderophore of *Pseudomonas putida* G4R. *Biochemistry*, **37**, 15965–15973.
Babcock,M., de Silva,D., Oaks,R., Davis-Kaplan,S., Jiralerspong,S., Montermini,L., Pandolfo,M. and Kaplan,J. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science*, **276**, 1709–1712.
Behrenfeld,M.J. and Kolber,Z.S. (1999) Widespread iron limitation of phytoplankton in the south pacific ocean. *Science*, **283**, 840–843.
Ben-Shachar,D., Livne,E., Spanier,I., Leenders,K.L. and Youdim,M.B. (1994) Typical and atypical neuroleptics induce alteration in blood–brain barrier and brain $^{59}FeCl_3$ uptake. *J. Neurochem*., **62**, 1112–1118.
Borjigin,J., Payne,A.S., Deng,J., Li,X., Wang,M.M., Ovodenko,B., Gitlin,J.D. and Snyder,S.H. (1999) A novel pineal night-specific ATPase encoded by the Wilson disease gene. *J. Neurosci*., **19**, 1018–1026.
Bothwell,T.H., Charlton,R.W. and Motulski,A.G. (1995) In Scriver,C.R., Beaudet,A.L., Sly,W.S. and Valle,D. (eds), *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York, NY, pp. 2237–2269.
Bull,P.C., Thomas,G.R., Rommens,J.M., Forbes,J.R. and Cox,D.W. (1993) The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nature Genet.*, **5**, 327–337.
Cellier,M., Prive,G., Belouchi,A., Kwan,T., Rodrigues,V., Chia,W. and Gros,P. (1995) Nramp defines a family of membrane proteins. *Proc. Natl Acad. Sci. USA*, **92**, 10089–10093.
Chan,J., Xing,Y., Magliozzo,R.S. and Bloom,B.R. (1992) Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med.*, **175**, 1111–1122.
Chen,X.Z., Zhu,T., Smith,D.E. and Hediger,M.A. (1999a) Stoichiometry and kinetics of the high-affinity H^+-coupled peptide transporter PepT2. *J. Biol. Chem.*, **274**, 2773–2779.
Culotta,V.C., Lin,S.J., Schmidt,P., Klomp,L.W., Casareno,R.L. and Gitlin,J. (1999) Intracellular pathways of copper trafficking in yeast and humans. *Adv. Exp. Med. Biol*., **448**, 247–254.
Dancis,A., Yuan,D.S., Haile,D., Askwith,C., Elde,D., Moehle,C., Kaplan,J. and Klausner,R.D. (1994a) Molecular characterization of a copper transport protein in *S.cerevisiae*: an unexpected role for copper in iron transport. *Cell*, **76**, 393–402.
Dancis,A., Haile,D., Yuan,D.S. and Klausner,R.D. (1994b) The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper and physiologic role in copper uptake. *J. Biol. Chem.*, **269**, 25660–25667.
Durr,G., Strayle,J., Plemper,R., Elbs,S., Klee,S.K., Catty,P., Wolf,D.H. and Rudolph,H.K. (1998) The medial-Golgi ion pump pmr1 supplies the yeast secretory pathway with Ca^{2+} and Mn^{2+} required for glycosylation, sorting and endoplasmic reticulum-associated protein degradation. *Mol. Biol. Cell*, **9**, 1149–1162.
Edwards,C.Q., Griffen,L.M., Goldgar,D., Drummond,C., Skolnick,M.H. and Kushner,J.P. (1988) Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N. Engl. J. Med.*, **318**, 1355–1362.
Eide,D.J. (1997) Molecular biology of iron and zinc uptake in eukaryotes. *Curr. Opin. Cell Biol.*, **9**, 573–577.
Eide,D.J. (1998) The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Annu. Rev. Nutr.*, **18**, 441–469.
Eskandari,S., Wright,E.M., Kreman,M., Starace,D.M. and Zampighi,G.A. (1998) Structural analysis of cloned plasma membrane proteins by freeze-fracture electron microscopy. *Proc. Natl Acad. Sci. USA*, **95**, 11235–11240.
Feder,J.N. *et al*. (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genet.*, **13**, 399–408.
Feder,J.N. *et al*. (1998) The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc. Natl Acad. Sci. USA*, **95**, 1472–1477.
Fisher,M., Zamir,A. and Pick,U. (1998) Iron uptake by the halotolerant alga *Dunaliella* is mediated by a plasma membrane transferrin. *J. Biol. Chem.*, **273**, 17553–17558.
Fleming,M.D. and Andrews,N.C. (1998) Mammalian iron transport: an unexpected link between metal homeostasis and host defense. *J. Lab. Clin. Med.*, **132**, 464–468.
Fleming,M.D., Romano,M.A., Su,M.A., Garrick,L.M., Garrick,M.D. and Andrews,N.C. (1998) Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc. Natl Acad. Sci. USA*, **95**, 1148–1153.
Fleming,M.D., Trenor,C.C.,III, Su,M.A., Foernzler,D., Beier,D.R., Dietrich,W.F. and Andrews,N.C. (1997) Microcytic anaemia mice have a mutation in *Nramp2*, a candidate iron transporter gene. *Nature Genet.*, **16**, 383–386.
Fleming,R.E. *et al*. (1999) Mechanism of increased iron absorption in a murine model of hereditary hemochromatosis: increased duodenal expression of the iron transporter DMT1. *Proc. Natl Acad. Sci. USA*, **96**, 3143–3148.
Fu,D., Beeler,T.J. and Dunn,T.M. (1995) Sequence, mapping and disruption of *CCC2*, a gene that cross-complements the Ca (2+)-sensitive phenotype of *csg1* mutants and encodes a P-type ATPase belonging to the Cu (2+)-ATPase subfamily. *Yeast*, **11**, 283–292.
Garrick,L.M., Dolan,K.G., Romano,M.A. and Garrick,M.D. (1999) Non-transferrin-bound iron uptake in Belgrade and normal rat erythroid cells. *J. Cell. Physiol.*, **178**, 349–358.
Gehring,N.H., Hentze,M.W. and Pantopoulos,K. (1999) Inactivation of both RNA binding and aconitase activities of iron regulatory protein-1 by quinone-induced oxidative stress. *J. Biol. Chem.*, **274**, 6219–6225.
Gruenheid,S., Canonne-Hergaux,F., Gauthier,S., Hackam,D.J., Grinstein,S. and Gros,P. (1999) The iron transport protein NRAMP2 is an integral membrane glycoprotein that colocalizes with transferrin in recycling endosomes. *J. Exp. Med.*, **189**, 831–841.
Gunshin,H., Mackenzie,B., Berger,U.V., Gunshin,Y., Romero,M.F., Boron,W.F., Nussberger,S., Gollan,J.L. and Hediger,M.A. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*, **388**, 482–488.
Han,O., Fleet,J.C. and Wood,R.J. (1999) Reciprocal regulation of HFE and Nramp2 gene expression by iron in human intestinal cells. *J. Nutr.*, **129**, 98–104.
Hawlitschek,G., Schneider,H., Schmidt,B., Tropschug,M., Hartl,F.-U. and Neupert,W. (1988) Mitochondrial protein import: identification of processing peptidase and of PEP, a processing enhancing protein. *Cell*, **53**, 795–806.
Hediger,M.A. (1997) Membrane permeability. The diversity of transmembrane transport processes. *Curr. Opin. Cell Biol.*, **9**, 543–546.
Howell,G.A., Welch,M.G. and Frederickson,C.J. (1984) Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature*, **308**, 736–738.
Hung,I.H., Suzuki,M., Yamaguchi,Y., Yuan,D.S., Klausner,R.D. and Gitlin,J.D. (1997) Biochemical characterization of the Wilson disease protein and functional expression in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **272**, 21461–21466.
Jordan,I. and Kaplan,J. (1994) The mammalian transferrin-independent iron transport system may involve a surface ferrireductase activity. *Biochem. J.*, **302**, 875–879.
Juel,C. and Halestrap,A.P. (1999) Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. *J. Physiol. (Lond.)*, **517**, 633–642.
Kampfenkel,K., Kushnir,S., Babiychuk,E., Inze,D. and Van Montagu,M. (1995) Molecular characterization of a putative *Arabidopsis thaliana* copper transporter and its yeast homologue. *J. Biol. Chem.*, **270**, 28479–28486.
Kaplan,J. and O'Halloran,T.V. (1996) Iron metabolism in eukaryotes: Mars and Venus at it again. *Science*, **271**, 1510–1512.
Kaufer,D., Friedman,A., Seidman,S. and Soreq,H. (1998) Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature*, **393**, 373–377.
Labbe,S., Zhu,Z. and Thiele,D.J. (1997) Copper-specific transcriptional repression of yeast genes encoding critical components in the copper transport pathway. *J. Biol. Chem.*, **272**, 15951–15958.
La Fontaine,S.L. *et al*. (1998) Correction of the copper transport defect

of Menkes patient fibroblasts by expression of the Menkes and Wilson ATPases. *J. Biol. Chem.*, **273**, 31375–31380.
Lesuisse,E., Simon-Casteras,M. and Labbe,P. (1998) Siderophore-mediated iron uptake in *Saccharomyces cerevisiae*: the *SIT1* gene encodes a ferrioxamine B permease that belongs to the major facilitator superfamily. *Microbiology*, **144**, 3455–3462.
Lewin,R.A. (1976) Prochlorophyta as a proposed new division of algae. *Nature*, **261**, 697–698.
Liu,X.F. and Culotta,V.C. (1999) Post-translation control of Nramp metal transport in yeast. Role of metal ions and the *BSD2* gene. *J. Biol. Chem.*, **274**, 4863–4868.
Liu,X.F., Supek,F., Nelson,N. and Culotta,V.C. (1997) Negative control of heavy metal uptake by the *Saccharomyces cerevisiae BSD2* gene. *J. Biol. Chem.*, **272**, 11763–11769.
Loo,D.D., Hazama,A., Supplisson,S., Turk,E. and Wright,E.M. (1993) Relaxation kinetics of the Na^+/glucose cotransporter. *Proc. Natl Acad. Sci. USA*, **90**, 5767–5771.
Loo,D.D., Hirayama,B.A., Gallardo,E.M., Lam,J.T., Turk,E. and Wright,E.M. (1998) Conformational changes couple Na^+ and glucose transport. *Proc. Natl Acad. Sci. USA*, **95**, 7789–7794.
Mackenzie,B., Loo,D.D., Fei,Y., Liu,W.J., Ganapathy,V., Leibach,F.H. and Wright,E.M. (1996) Mechanisms of the human intestinal H^+-coupled oligopeptide transporter hPEPT1. *J. Biol. Chem.*, **271**, 5430–5437.
Maeda,M., Hamano,K., Hirano,Y., Suzuki,M., Takahashi,E., Terada,T., Futai,M. and Sato,R. (1998) Structures of P-type transporting ATPases and chromosomal locations of their genes. *Cell Struct. Funct.*, **23**, 315–323.
Mager,S., Naeve,J., Quick,M., Labarca,C., Davidson,N. and Lester,H.A. (1993) Steady states, charge movements and rates for a cloned GABA transporter expressed in *Xenopus* oocytes. *Neuron*, **10**, 177–188.
Mager,S., Kleinberger-Doron,N., Keshet,G.I., Davidson,N., Kanner,B.I. and Lester,H.A. (1996) Ion binding and permeation at the GABA transporter GAT1. *J. Neurosci.*, **16**, 5405–5414.
McMahon,R.J. and Cousins,R.J. (1998) Mammalian zinc transporters. *J. Nutr.*, **128**, 667–670.
Moore,D.F. and Sears,D.A. (1994) Pica, iron deficiency and the medical history. *Am. J. Med.*, **97**, 390–393.
Morris,C.M., Keith,A.B., Edwardson,J.A. and Pullen,R.G. (1992) Uptake and distribution of iron and transferrin in the adult rat brain. *J. Neurochem.*, **59**, 300–306.
Murphy,V.A., Wadhwani,K.C., Smith,Q.R. and Rapoport,S.I. (1991) Saturable transport of manganese (II) across the rat blood–brain barrier. *J. Neurochem.*, **57**, 948–954.
Nelson,N. (1992) Evolution of organellar proton-ATPases. *Biochim. Biophys. Acta*, **1100**, 109–124.
Nelson,N. (1998) The family of Na^+/Cl^- neurotransmitter transporters. *J. Neurochem.*, **71**, 1785–1803.
Nelson,N. and Harvey,W.R. (1999) Vacuolar and plasma membrane V-ATPases. *Phys. Rev.*, **79**, 361–385.
Okorokov,L.A., Tanner,W. and Lehle,L. (1993) A novel primary Ca (2+)-transport system from *Saccharomyces cerevisiae*. *Eur. J. Biochem.*, **216**, 573–577.
Okorokov,L.A. and Lehle,L. (1998) Ca^{2+}-ATPases of *Saccharomyces cerevisiae*: diversity and possible role in protein sorting. *FEMS Microbiol. Lett.*, **162**, 83–91.
Ooi,C.E., Rabinovich,E., Dancis,A., Bonifacino,J.S. and Klausner,R.D. (1996) Copper-dependent degradation of the *Saccharomyces cerevisiae* plasma membrane copper transporter Ctr1p in the apparent absence of endocytosis. *EMBO J.*, **15**, 3515–3523.
Orgad,S., Nelson,H., Segal,D. and Nelson,N. (1998) Metal ions suppress the abnormal taste behavior of the *Drosophila* mutant *malvolio*. *J. Exp. Biol.*, **201**, 115–120.
Paidhungat,M. and Garrett,S. (1998a) Cdc1 and the vacuole coordinately regulate Mn^{2+} homeostasis in the yeast *Saccharomyces cerevisiae*. *Genetics*, **148**, 1787–1798.
Paidhungat,M. and Garrett,S. (1998b) Cdc1 is required for growth and Mn^{2+} regulation in *Saccharomyces cerevisiae*. *Genetics*, **148**, 1777–1786.
Parkkila,S., Waheed,A., Britton,R.S., Bacon,B.R., Zhou,X.Y., Tomatsu,S., Fleming,R.E. and Sly,W.S. (1997) Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proc. Natl Acad. Sci. USA*, **94**, 13198–13202.
Peters,S., Koh,J. and Choi,D.W. (1987) Zinc selectively blocks the action of *N*-methyl-D-aspartate on cortical neurons. *Science*, **236**, 589–593.
Petris,M.J., Mercer,J.F., Culvenor,J.G., Lockhart,P., Gleeson,P.A. and Camakaris,J. (1996) Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. *EMBO J.*, **15**, 6084–6095.
Petrukhin,K. *et al.* (1993) Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nature Genet.*, **5**, 338–343.
Phung,L.T., Ajlani,G. and Haselkorn,R. (1994) P-type ATPase from cyanobacterium *Synechococcus* 7942 related to the human Menkes and Wilson disease gene products. *Proc. Natl Acad. Sci. USA*, **91**, 9651–9654.
Pinner,E., Gruenheid,S., Raymond,M. and Gros,P. (1997) Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance-associated macrophage protein family. *J. Biol. Chem.*, **272**, 28933–28938.
Plant,J.E., Blackwell,J.M., O'Brien,A.D., Bradley,D.J. and Glynn,A.A. (1982) Are the *Lsh* and *Ity* disease resistance genes at one locus on mouse chromosome 1? *Nature*, **297**, 510–511.
Pollock,R.A., Hartl,F.-U., Cheng,M.Y., Ostermann,J., Horwich,A. and Neupert,W. (1988) The processing peptidase of yeast mitochondria: the two co-operating components MPP and PEP are structurally related. *EMBO J.*, **7**, 3493–3500.
Rabin,O., Hegedus,L., Bourre,J.M. and Smith,Q.R. (1993) Rapid brain uptake of manganese (II) across the blood–brain barrier. *J. Neurochem.*, **61**, 509–517.
Radisky,D.C. and Kaplan,J. (1999) Regulation of transition metal transport across the yeast plasma membrane. *J. Biol. Chem.*, **274**, 4481–4484.
Rodrigues,V., Cheah,P.Y., Ray,K. and Chia,W. (1995) Malvolio, the *Drosophila* homologue of mouse NRAMP-1 (Bcg), is expressed in macrophages and in the nervous system and is required for normal taste behaviour. *EMBO J.*, **14**, 3007–3020.
Roetto,A. *et al*. (1999) Juvenile hemochromatosis locus maps to chromosome 1q. *Am. J. Hum. Genet.*, **64**, 1388–1393.
Roy,C.N., Penny,D.M., Feder,J.N. and Enns,C.A. (1999) The hereditary hemochromatosis protein, HFE, specifically regulates transferrin-mediated iron uptake in HeLa cells. *J. Biol. Chem.*, **274**, 9022–9028.
Rudolph,H.K., Antebi,A., Fink,G.R., Buckley,C.M., Dorman,T.E., LeVitre,J., Davidow,L.S., Mao,J.I. and Moir,D.T. (1989) The yeast secretory pathway is perturbed by mutations in PMR1, a member of a Ca^{2+} ATPase family. *Cell*, **58**, 133–145.
Schaefer,M. and Gitlin,J.D. (1999) Genetic disorders of membrane transport. IV. Wilson's disease and Menkes disease. *Am. J. Physiol.*, **276**, G311–G314.
Segal,A.W. and Abo,A. (1993) The biochemical basis of the NADPH oxidase of phagocytes. *Trends Biochem. Sci.*, **18**, 43–47.
Seguela,P., Haghighi,A., Soghomonian,J.J. and Cooper,E. (1996) A novel neuronal P2x ATP receptor ion channel with widespread distribution in the brain. *J. Neurosci.*, **16**, 448–455.
Sensi,S.L., Canzoniero,L.M., Yu,S.P., Ying,H.S., Koh,J.Y., Kerchner,G.A. and Choi,D.W. (1997) Measurement of intracellular free zinc in living cortical neurons: routes of entry. *J. Neurosci.*, **17**, 9554–9564.
Shatwell,K.P., Dancis,A., Cross,A.R., Klausner,R.D. and Segal,A.W. (1996) The FRE1 ferric reductase of *Saccharomyces cerevisiae* is a cytochrome b similar to that of NADPH oxidase. *J. Biol. Chem.*, **271**, 14240–14244.
Steel,A., Nussberger,S., Romero,M.F., Boron,W.F., Boyd,C.A. and Hediger,M.A. (1997) Stoichiometry and pH dependence of the rabbit proton-dependent oligopeptide transporter PepT1. *J. Physiol.*, **498**, 563–569.
Supek,F., Supekova,L., Nerlson,H. and Nelson,N. (1996) A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proc. Natl Acad. Sci. USA*, **93**, 5105–5110.
Supek,F., Supekova,L., Nelson,H. and Nelson,N. (1997) Function of metal-ion homeostasis in cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain functions. *J. Exp. Biol.*, **200**, 321–330.
van Gelder,W., Huijskes-Heins,M.I., Cleton-Soeteman,M.I., van Dijk,J.P. and van Eijk,H.G. (1998) Iron uptake in blood–brain barrier endothelial cells cultured in iron-depleted and iron-enriched media. *J. Neurochem.*, **71**, 1134–1140.
Vidal,S.M., Malo,D., Vogan,K., Skamene,E. and Gros,P. (1993) Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell*, **73**, 469–485.

Vidal,S., Tremblay,M.L., Govoni,G., Gauthier,S., Sebastiani,G., Malo,D., Skamene,E., Olivier,M., Jothy,S. and Gros,P. (1995) The *Ity/Lsh/Bcg* locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the *Nramp1* gene. *J. Exp. Med.*, **182**, 655–666.

Vulpe,C., Levinson,B., Whitney,S., Packman,S. and Gitschier,J. (1993) Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nature Genet.*, **3**, 7–13.

Waheed,A., Parkkila,S., Saarnio,J., Fleming,R.E., Zhou,X.Y., Tomatsu,S., Britton,R.S., Bacon,B.R. and Sly,W.S. (1999) Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. *Proc. Natl Acad. Sci. USA*, **96**, 1579–1584.

West,A.H., Clark,D.J., Martin,J., Neupert,W., Hartl,F.-U. and Horwich,A.L. (1992) Two related genes encoding extremely hydrophobic proteins suppress a lethal mutation in the yeast mitochondrial processing enhancing protein. *J. Biol. Chem.*, **267**, 24625–24633.

Westbrook,G.L. and Mayer,M.L. (1987) Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature*, **328**, 640–643.

Witte,C., Jensen,R.E., Yaffe,M.P. and Schatz,G. (1988) *MAS1*, a gene essential for yeast mitochondrial assembly, encodes a subunit of the mitochondrial processing protease. *EMBO J.*, **7**, 1439–1447.

Wright,E.M., Loo,D.D., Panayotova-Heiermann,M., Lostao,M.P., Hirayama,B.H., Mackenzie,B., Boorer,K. and Zampighi,G. (1994) 'Active' sugar transport in eukaryotes. *J. Exp. Biol.*, **196**, 197–212.

Yang,M., Jensen,R.E., Yaffe,M.P., Oppliger,W. and Schatz,G. (1988) Import of proteins into yeast mitochondria: the purified matrix processing protease contains two subunits which are encoded by the nuclear *MAS*1 and *MAS2* genes. *EMBO J.*, **7**, 3857–3862.

Yuan,D.S., Dancis,A. and Klausner,R.D. (1997) Restriction of copper export in *Saccharomyces cerevisiae* to a late Golgi or post-Golgi compartment in the secretory pathway. *J. Biol. Chem.*, **272**, 25787–25793.

Zhou,B. and Gitschier,J. (1997) *hCTR1*: a human gene for copper uptake identified by complementation in yeast. *Proc. Natl Acad. Sci. USA*, **94**, 7481–7486.

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