

# The Many Faces of the Copper Metabolism Protein MURRI/COMMD1

P. DE BIE, B. VAN DE SLUIS, L. KLOMP, AND C. WIJMENGA

From the Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Center, 3508 TA Utrecht, The Netherlands (de Bie, van de Sluis, Wijmenga); and the Laboratory of Metabolic and Endocrine Diseases, University Medical Center, 3584 EA Utrecht, The Netherlands (de Bie, van de Sluis, Klomp).

Address correspondence to Cisca Wijmenga at the Complex Genetics Section, Department of Biomedical Genetics, Str. 2.117, University Medical Center Utrecht, PO Box 85060, 3508 AB Utrecht, The Netherlands; or e-mail: t.n.wijmenga@med.uu.nl.

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## Abstract

Copper is an essential transition metal but is toxic in excess; therefore, its metabolism needs to be tightly regulated. Defects in the regulation of copper can lead to various disorders characterized by copper deficiency or copper excess. Recently, we characterized the *COMMD1* (previously *MURR1*) gene as the defective gene in canine copper toxicosis. The molecular functions of *COMMD1* remain unknown, but significant progress has been made in identifying the cellular processes in which *COMMD1* participates, through the identification of proteins interacting with *COMMD1*. This review discusses how *COMMD1* functions as a regulator of not only copper homeostasis but also sodium transport and the NF- $\kappa$ B signaling pathway. We outline the possible mechanisms through which *COMMD1* exerts these newly identified functions.

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Copper is an essential trace element for all living organisms, as it functions as a cofactor for a number of cuproenzymes. Copper's ability to exist in two oxidation states enables it to be an important cofactor for various enzymes that require redox chemistry for their function. These enzymes include Cu/Zn superoxide dismutase, which protects the cell against free radicals, and the cytochrome C oxidase complex, which is an essential component of the mitochondrial respiratory chain. In contrast to its necessity, its ability to shift between oxidation states renders copper highly toxic when in excess, owing to its potential to facilitate the generation of reactive oxygen species; therefore, a tight regulation of copper homeostasis is required. Under normal homeostatic conditions in mammals, the uptake of copper through the intestine ensures that essential needs are met, whereas excretion through the bile prevents toxicity (Wijmenga and Klomp 2004). Various genetic diseases of copper metabolism are characterized by either depletion or accumulation of copper (see Table 1), underlining the importance of a tight regulation.

Menkes disease (Menkes et al. 1962) is characterized by a general copper deficiency due to malabsorption of copper from the diet, and it results in early retardation in growth, peculiar ("kinky") hair, and focal cerebral and cerebellar degeneration. The disease is caused by mutations in the *ATP7A* gene (Chelly et al. 1993; Mercer et al. 1993; Vulpe et al. 1993), which encodes a copper translocating P-type ATPase. Wilson

disease is a copper-overload disorder (Wilson 1912) caused by mutations in the *ATP7B* gene (Bull et al. 1993; Tanzi et al. 1993; Yamaguchi et al. 1993). In Wilson disease copper accumulates in the liver and brain, causing extensive hepatic and neurological abnormalities. Other, non-Wilsonian forms of hepatic copper-overload syndromes have been described (Muller et al. 2004; Wijmenga et al. 1998), including Indian childhood cirrhosis (Tanner 1998), endemic Tyrolean infantile cirrhosis (Muller et al. 1996), and sporadic cases occurring worldwide grouped together as idiopathic copper toxicosis (Muller et al. 1998; Scheinberg and Sternlieb 1996). These non-Wilsonian copper-overload disorders are fatal at an early age because of liver failure as a consequence of chronic liver cirrhosis. Many of these disorders can be classified as ecogenetic disorders in the sense that both an excessive copper intake and a genetic defect underlie their pathology (Muller et al. 1996; Muller et al. 1999; Tanner 1998).

## Copper Toxicosis in Bedlington Terriers

One particular form of copper overload in dogs has long been considered an excellent model for studying hepatic copper overload and for gaining insight into the etiology of non-Wilsonian copper-overload disorders. Canine copper toxicosis is an autosomal recessive disorder, with a high frequency in Bedlington terriers (Hardy et al. 1975). Biliary copper excretion is markedly reduced in affected dogs (Su et al.

**Table 1.** Lists of human and animal disorders characterized by a defective copper homeostasis

	Defective gene	OMIM	References
<b>Human copper deficiency or overload disorders</b>			
Menkes disease	<i>ATP7A</i>	309400	Chelly et al. 1993; Mercer et al. 1993; Vulpe et al. 1993
Occipital horn syndrome	<i>ATP7A</i>	304150	Kaler et al. 1994
Wilson disease	<i>ATP7B</i>	277900	Bull et al. 1993; Tanzi et al. 1993; Yamaguchi et al. 1993
Indian childhood cirrhosis	?	215600	Tanner 1998; Tanner et al. 1979
Endemic Tyrolean infantile cirrhosis	?	215600	Muller et al. 1996
Idiopathic copper toxicosis	?	215600	Muller et al. 1998; Scheinberg and Sternlieb 1996
<b>Animal models for copper deficiency or overload</b>			
Canine copper toxicosis	<i>COMMD1</i>	607238	van de Sluis et al. 2002
Mottled mouse	<i>Atp7a</i>	309400	Cecchi et al. 1997; Reed and Boyd 1997
Brindled mouse	<i>Atp7a</i>	309400	Grimes et al. 1997
LEC rat	<i>Atp7b</i>	277900	Wu et al. 1994
Toxic milk mouse	<i>Atp7b</i>	277900	Theophilos et al. 1996
North Ronaldsay sheep	?	—	MacLachlan and Johnston 1982

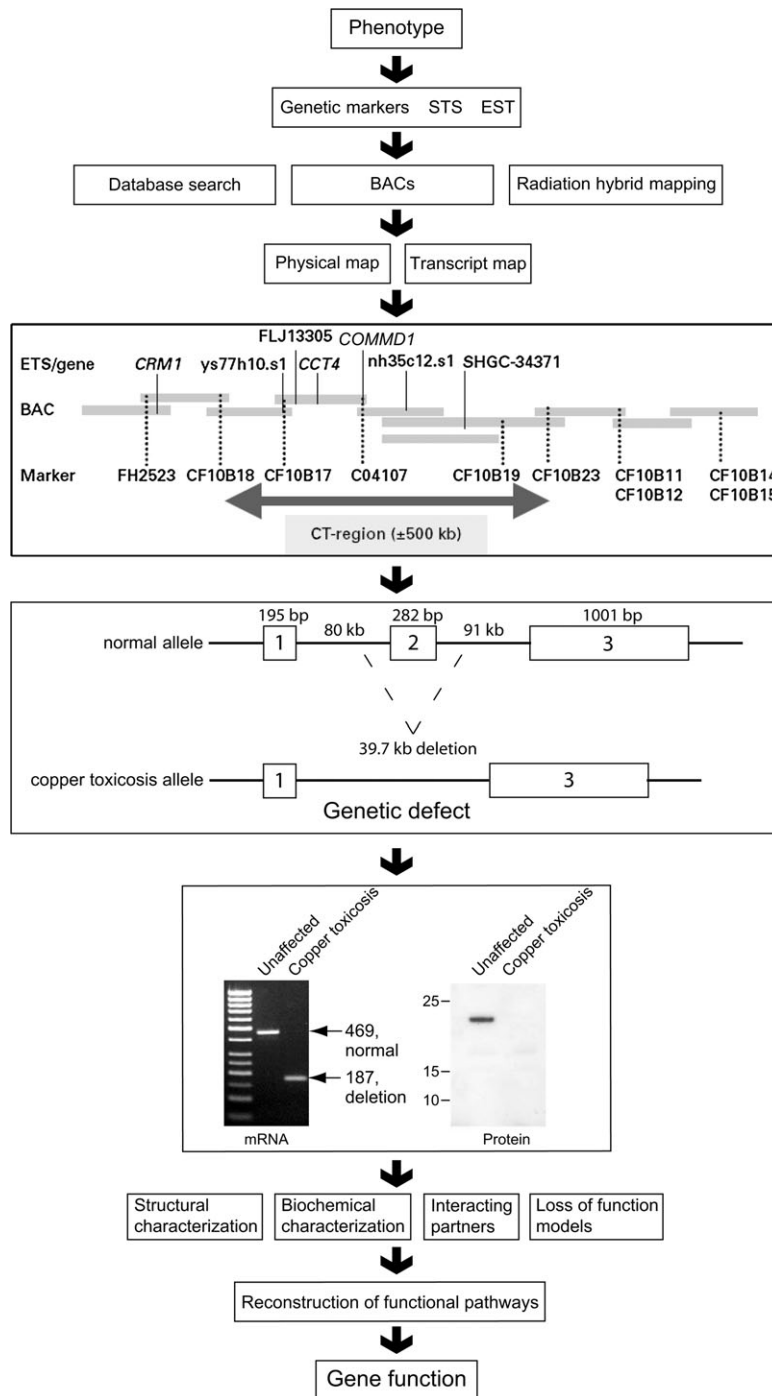
OMIM = Online Mendelian Inheritance in Man (catalog of heritable human traits; see [www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM)); LEC = Long-Evans Cinnamon.

1982), and as a result copper accumulates in lysosomes of hepatocytes, eventually leading to liver cirrhosis and chronic hepatitis (Twedt et al. 1979). Linkage analysis studies have shown that the copper toxicosis locus was closely associated with the C04107 microsatellite marker (Yuzbasiyan-Gurkan et al. 1997). Mapping of the C04107 marker to canine chromosome 10q26, a region syntenic to human chromosome 2p13–p16, excluded known copper-related candidate genes, such as *ATP7B* and *ATOX1*, based on their chromosomal localization (Dagenais et al. 1999; Nanji and Cox 1999; van de Sluis et al. 1999, 2001). To positionally clone the copper toxicosis gene, a physical map of the copper toxicosis region was constructed using a bacterial artificial clone contig and radiation hybrid mapping (van de Sluis et al. 2000, 2002). Homozygosity and linkage disequilibrium mapping further confined the copper toxicosis region to approximately 500 kb, containing 16 putative coding sequences, of which 6 were mapped genes (van de Sluis et al. 2005). A homozygous 39.7 kb genomic deletion encompassing exon 2 of the *COMMD1* (previously *MURR1*) gene was subsequently identified by positional cloning as the genetic cause of canine copper toxicosis (Forman et al. in press; van de Sluis et al. 2002). The *COMMD1* gene was initially described for its proximity to the imprinted *U2af1-rs1* gene in the mouse genome (Nabetani et al. 1997); its canine ortholog consists of three exons and encodes a 188–amino acid protein with a predicted  $M_r$  of 23 kDa (Klomp et al. 2003; van de Sluis et al. 2002). Although the mutant allele is predicted to lead to an in-frame deletion in the *COMMD1* transcript, resulting in a truncated protein of 94 amino acids, no full-length nor truncated *COMMD1* protein is detectable in liver homogenates of Bedlington terriers affected with copper toxicosis (Klomp et al. 2003), suggesting that a complete loss of function of *COMMD1* underlies the canine copper toxicosis pathology (summarized in Figure 1).

Although the C04107 marker is located in intron 1 of the *COMMD1* gene at approximately 13.5 kb from the proxi-

mal breakpoint of the *COMMD1* deletion (Forman et al., in press), several different haplotypes composing the copper toxicosis locus have been described, implicating a new level of complexity in the disease (Coronado et al. 2003; Haywood et al. 2001; Hyun et al. 2004; van de Sluis et al. 2003; Yuzbasiyan-Gurkan et al. 1997). One notable group includes affected dogs that do not contain the *COMMD1* exon 2 deletion nor any other mutation in the *COMMD1* coding region (Coronado et al. 2003; Hyun et al. 2004), which may suggest that, as yet, unidentified mutations in regulatory elements of the *COMMD1* gene underlie the disorder in these dogs. Alternatively, these dogs could have mutations in entirely different genes, for which the other *COMMD* proteins (discussed later) are potentially interesting candidates.

*COMMD1* mRNA is expressed abundantly in liver, but is also readily detected in other tissues (van de Sluis et al. 2002). Consistent with this observation, an ubiquitous expression pattern of *COMMD1* protein has been detected in murine tissues and various human cell lines using a polyclonal antiserum raised against *COMMD1* (Klomp et al. 2003). Although *COMMD1* is strongly conserved throughout evolution, it seems to be confined to higher eukaryotes (i.e., bile-containing organisms). The *COMMD1* sequence contains no apparent copper-binding motifs, but it does contain a novel conserved domain known as the copper metabolism gene *MURR1* (COMM) domain (Burstein et al. 2005). The COMM domain is leucine rich, consists of roughly 85 amino acids, and is present in nine other human genes (*COMMD2-10*). Identification of *COMMD* led to reannotation of *MURR1* to *COMMD1*. Strikingly, all 10 *COMMD* proteins can be found in complex with *COMMD1* (Burstein et al. 2005), although the exact composition of these *COMMD1*-*COMMD* complexes in vivo still needs to be determined. The exact molecular function of *COMMD1* remains unknown, but significant progress has recently been made in identifying several cellular processes in which



**Figure 1.** Schematic overview of positional cloning strategy employed in cloning the *COMMD1* gene. Genetic mapping was used to map the disease gene onto the dog genome (Yuzbasiyan-Gurkan et al. 1997). The map location was determined by comparative mapping (van de Sluis et al. 1999). Radiation hybrid mapping was used to construct a physical map (van de Sluis et al. 2000). A bacterial artificial chromosome (BAC) contig was constructed, and new polymorphic markers were isolated from the BAC clones to narrow down the canine copper toxicosis gene region further by genetic mapping. Haplotype sharing revealed a region of approximately 500 kb shared by all affected animals so that genes from this region were subjected to mutation analysis by sequencing. The *COMMD1* gene was found to be mutated in affected Bedlington terriers, which was associated with a short RNA product (van de Sluis et al. 2002). The mutation results in a loss of function because no protein can be detected in the livers of affected dogs (Klomp et al. 2003). Further functional characterization of *COMMD1* will be based on characterizing biochemical features, protein-protein interactions involving *COMMD1*, and loss of function models. STS = sequence tagged site; EST = expressed sequence tag.

COMMD1 participates, through the identification of proteins interacting with COMMD1.

## COMMD1 as a Regulator of Copper Homeostasis

The liver plays a key role in the excretion of copper from the human body. Within the liver, copper excretion is critically dependent on the function of ATP7B, a copper translocating P-type ATPase that is structurally and functionally homologous to ATP7A. ATP7B contains six tandemly repeated copper binding sites in its amino terminus. On entering the cell through the copper transporter 1 (CTR1; Zhou and Gitschier 1997), copper is delivered to the Golgi compartment by the copper chaperone ATOX1 (Klomp et al. 1997). ATOX1 is able to bind copper and undergoes a transient copper-dependent association with ATP7B (Hamza et al. 1999; Larin et al. 1999; van Dongen et al. 2004). Under physiological conditions ATP7B resides in the trans-Golgi network. When copper levels rise, however, ATP7B translocates to a diffuse vesicular compartment (Hung et al. 1997) from which copper is secreted from the cell in an unknown manner. A similar copper-dependent trafficking has been observed for ATP7A, which translocates to a vesicular compartment and the plasma membrane upon elevation of copper levels (Petris et al. 1996). Molecular mechanisms of the copper-dependent intracellular trafficking of ATP7B and ATP7A are largely unknown, but trafficking of ATP7A is markedly impaired in cells isolated from *ATOX1*<sup>-/-</sup> mice (Hamza et al. 2003). It has also been shown that mutations that impair the interaction of ATP7B with ATOX1 are associated with Wilson disease (Hamza et al. 1999), suggesting that ATOX1 plays an essential role in this mechanism.

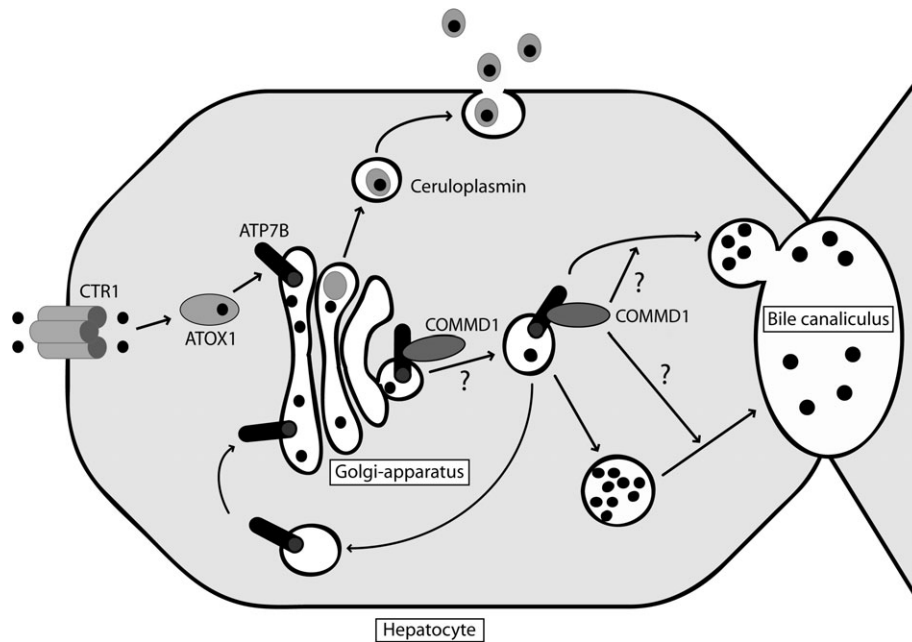
COMMD1 also plays a critical role in copper excretion, as can be deduced from the hepatic copper overload and reduced biliary excretion phenotype in Bedlington terriers with a *COMMD1* deletion. Recent studies have shown that COMMD1 is able to bind to the N-terminal copper binding region of ATP7B but not that of ATP7A (Tao et al. 2003). These data suggest that COMMD1 and ATP7B cooperate in the excretion of copper from the hepatocyte and that abolishment of the interaction between these proteins underlies the pathophysiology of canine copper toxicosis. Copper transport to the Golgi compartment is unaffected in canine copper toxicosis, as the dogs exhibit normal serum ceruloplasmin levels, suggesting that COMMD1 possibly participates in the ATP7B-mediated transport of copper from the trans-Golgi to the bile canaliculus. Because the dogs show massive copper accumulation in lysosomes of the hepatocyte, a possible role for COMMD1 might be to facilitate degranulation of lysosomal contents into the bile (Figure 2). This model is consistent with the observation that COMMD1 localizes to a vesicular compartment showing partial overlap with the transferrin receptor and CD63, which are markers for early endosomes and lysosomes, respectively (Klomp et al. 2003).

Presently, no mutations in human *COMMD1* in patients with Wilson disease have been described, although heterozygosity for a silent missense mutation in *COMMD1* is possibly associated with an earlier onset of the disorder in patients with known *ATP7B* mutations (Stuehler et al. 2004). In addition, no mutations in *COMMD1* were detected among non-Wilsonian copper-overload patients in a cohort containing 12 Indian childhood cirrhosis patients, 1 endemic Tyrolean infantile cirrhosis patient, and 10 idiopathic copper toxicosis patients (Muller et al. 2003), excluding canine copper toxicosis as a genetic model for these disorders. Transient knockdown of COMMD1 in *HEK293* leads to increased cellular copper levels (Burstein et al. 2004), supporting the view of COMMD1 as a regulator of copper homeostasis. Further studies of ATP7B trafficking and functioning in COMMD1-deficient systems might enhance our understanding of COMMD1 functioning in the ATP7B-mediated copper-excretion pathway.

Recent studies imply that cellular COMMD1 levels are regulated by the X-linked inhibitor of apoptosis (XIAP). As an E3 ubiquitin ligase, XIAP ubiquitinates COMMD1, thereby targeting it for proteasomal degradation (Burstein et al. 2004). Interestingly, it was observed that fibroblasts and liver tissue of *XIAP*<sup>-/-</sup> mice display a reduced copper content (Burstein et al. 2004), indicating that XIAP regulates copper homeostasis, possibly by mediating COMMD1 levels. Several proteins that are either involved in copper homeostasis or that require copper for their function are regulated at their protein levels in response to altered intracellular copper levels, including hephaestin (Nittis and Gitlin 2004), Cu/Zn superoxide dismutase (Bertinato and L'Abbe 2003), and the copper transporter CTR1 (Guo et al. 2004; Ooi et al. 1996; Petris et al. 2003). The existence of copper-dependent internalization and degradation of CTR1 is, however, still under debate (Eisses and Kaplan 2005; Klomp et al. 2002). It remains to be investigated if copper regulates XIAP activity, thereby providing a mechanism for copper-regulated COMMD1 degradation. However, it has already been shown that COMMD1 levels remain constant under changing copper levels (Klomp et al. 2003), suggesting that copper dependence of XIAP-mediated COMMD1 ubiquitination would be unlikely.

## COMMD1 as a Regulator of Sodium Uptake

The amiloride-sensitive epithelial sodium channel (ENaC) constitutes the rate-limiting step for sodium reabsorption in epithelial cells that line the distal part of the renal tubule, the distal colon, the duct of several exocrine glands, and the lung (Canessa et al. 1994). ENaC consists of three similar subunits, of which the  $\alpha$ -,  $\beta$ -, and the  $\gamma$ -subunits have initially been characterized (Canessa et al. 1994; McDonald et al. 1995). An additional  $\delta$ -ENaC subunit was later identified that cooperates with the  $\beta$ - and  $\gamma$ -ENaC subunits to attain an amiloride-induced sodium current (Waldmann et al. 1995). COMMD1 has been reported to interact with the C-terminus



**Figure 2.** Overview of the ATP7B-mediated copper export pathway in the hepatocyte. Upon entering the cell through the copper transporter CTR1, copper is delivered to ATP7B in the golgi apparatus by the copper chaperone ATOX1. In the golgi apparatus copper is incorporated in various cuproenzymes including ceruloplasmin. When copper levels in the cell rise, ATP7B redistributes to a vesicular compartment. Upon relocation of ATP7B, copper is excreted from the hepatocyte through the bile via an unknown mechanism that probably involves COMMD1.

of  $\delta$ -ENaC (Biasio et al. 2003) and has been detected in complex with  $\beta$ - and  $\gamma$ -ENaC but not with  $\alpha$ -ENaC. Coexpression of COMMD1 with  $\delta$ -,  $\beta$ -, and  $\gamma$ -ENaC inhibits the amelioride-induced sodium current, which depends on the C-terminus of  $\delta$ -ENaC as demonstrated by deletion mapping. An inhibitory effect of COMMD1 on the  $\alpha$ -,  $\beta$ -,  $\gamma$ -ENaC was also observed but to a lesser extent (Biasio et al. 2003). How COMMD1 inhibits  $\alpha$ -,  $\beta$ -, and  $\gamma$ -ENaC activity is unclear, but it has been postulated that this occurs through binding to the  $\beta$ - or  $\gamma$ -ENaC subunits, although a direct interaction between COMMD1 and these subunits independent of  $\delta$ -ENaC has not been demonstrated. These findings shed a new and unexpected light on the cellular functions of COMMD1.

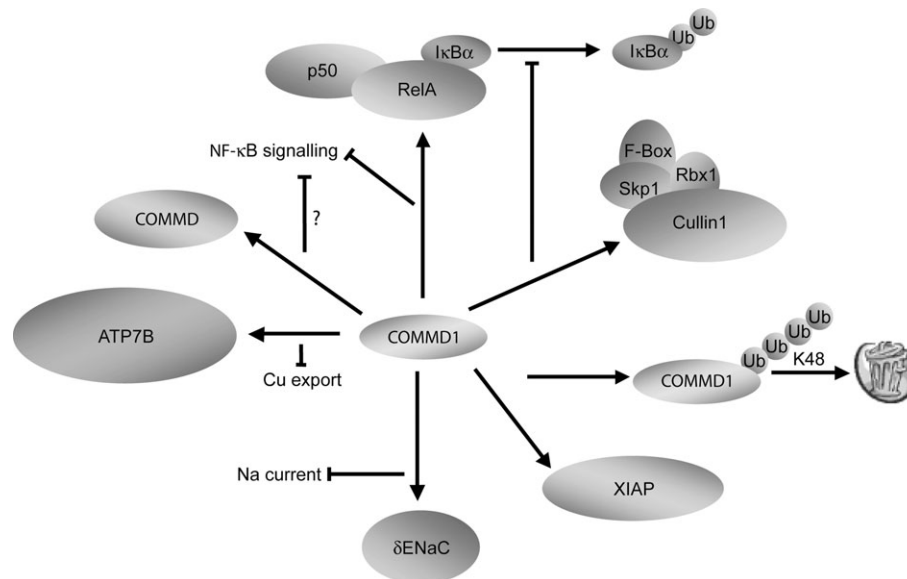
For correct functioning of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -ENaC, it trafficks from the Golgi compartment to the cell membrane after glycosylation on all three subunits (Hanwell et al. 2002, Rotin et al. 2001). It has been suggested that on the cell membrane,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -ENaC stability is regulated by Nedd4-mediated ubiquitination and clathrin-mediated endocytosis (Rotin et al. 2001). Relatively little is known about the exact mechanisms of ENaC trafficking and the possibility of  $\delta$ -,  $\beta$ -, and  $\gamma$ -ENaC trafficking still needs to be investigated. Nevertheless, based on the interactions of COMMD1 with both ATP7B and  $\delta$ -,  $\beta$ -,  $\gamma$ -ENaC, and the fact that both transporters are possibly regulated in their activity by intracellular trafficking, we could speculate that COMMD1 plays a key role in regulating this process. Studies determining the role of COMMD1 on sub-cellular localization and trafficking of both ATP7B and

$\delta$ -,  $\beta$ -,  $\gamma$ -ENaC need to be performed to investigate this hypothesis.

Copper uptake in fish gills has been shown to be inhibited by elevated sodium levels (Pyle et al. 2003) and vice versa (Laurén and McDonald 1987; Reid and McDonald 1988), linking sodium transport to copper transport. The exact link between copper and sodium transport needs further characterization but has been postulated to involve ENaC (Handy et al. 2002). Considering the critical role of COMMD1 in copper metabolism, we could speculate that regulation of ENaC activity by COMMD1 might provide the link between sodium and copper transport.

### COMMD1 as a Regulator of NF- $\kappa$ B Signaling

The nuclear factor- $\kappa$ B (NF- $\kappa$ B) complex plays an important role in the transcriptional regulation of a wide array of genes, the majority of which are involved in immune and stress responses (Pahl 1999). In unstimulated cells, different NF- $\kappa$ B proteins exist as either homo- or heterodimers bound to NF- $\kappa$ B inhibitor (I $\kappa$ B) proteins (Hayden and Ghosh 2004). NF- $\kappa$ B proteins are characterized by the presence of the Rel homology domain that is responsible for their dimerization, their interaction with I $\kappa$ B proteins, and their DNA binding (Kumar et al. 1992; Latimer et al. 1998; Logeat et al. 1991; Ruben et al. 1992). I $\kappa$ B proteins act as inhibitors of NF- $\kappa$ B signaling by binding to the



**Figure 3.** Schematic overview of different COMMD1 protein-protein interactions and their functional implications. Known protein-protein interactions involving COMMD1 are summarized in this figure. Through identification of its molecular partners, COMMD1 can be placed in several biological pathways. Exact biological mechanisms, however, are still lacking, and further research is required.

NF- $\kappa$ B dimers and thereby masking the nuclear localization sequence of the NF- $\kappa$ B subunit RelA; as a result, the NF- $\kappa$ B complex is retained in the cytoplasm (Huxford et al. 1998; Jacobs and Harrison 1998; Yamamoto and Gaynor 2004). In the classical pathway of NF- $\kappa$ B activation, I $\kappa$ B proteins are phosphorylated by activated I $\kappa$ B kinases, which targets I $\kappa$ B proteins for ubiquitination and subsequent proteasomal degradation (Chen et al. 1995; DiDonato et al. 1996; Scherer et al. 1995). After I $\kappa$ B degradation the nuclear localization signal of NF- $\kappa$ B is unmasked, resulting in nuclear translocation and transcriptional activation of  $\kappa$ B target genes.

The identification of COMMD1 as interacting partner for XIAP, an activator of NF- $\kappa$ B signaling, led to investigations indicating that COMMD1, as well as other COMMD proteins, can be a potent repressor of NF- $\kappa$ B activation induced by various stimuli in several different cell lines (Burstein et al. 2005; Ganesh et al. 2003). COMMD1 was shown to interact with several NF- $\kappa$ B subunits as well as with I $\kappa$ B $\alpha$ , indicating that COMMD1 directly participates in the NF- $\kappa$ B pathway (Burstein et al. 2005; Ganesh et al. 2003).

Ubiquitination of I $\kappa$ B $\alpha$  upon activation of the classical NF- $\kappa$ B pathway is mediated by the Skp1-Cullin1-F-box-protein (SCF) ubiquitin ligase complex (Fuchs et al. 1999; Hatakeyama et al. 1999; Kroll et al. 1999; Tan et al. 1999; Winston et al. 1999). The SCF complex consists of the core subunits Cullin1, RBX1, and Skp1. The last acts to recruit F-box-containing proteins that determine target specificity of the SCF complex (Cardozo and Pagano 2004); siRNA-mediated knockdown of COMMD1 leads to decreased I $\kappa$ B $\alpha$

levels, suggesting that COMMD1 has a protective effect on I $\kappa$ B $\alpha$ . An interaction between COMMD1 and the SCF subunit Cullin1 has been detected (Ganesh et al. 2003), which led to the hypothesis that COMMD1 protects I $\kappa$ B $\alpha$  from proteasomal degradation by preventing ubiquitination of I $\kappa$ B $\alpha$  by the SCF complex (Ganesh et al. 2003; Greene 2004). However, as this hypothesis suggests, due to increased I $\kappa$ B $\alpha$  levels, NF- $\kappa$ B would be retained in the cytoplasm upon overexpression of COMMD1, no impairment of nuclear translocation of NF- $\kappa$ B upon stimulation with TNF is observed (Burstein et al. 2005). This suggests that other mechanisms of NF- $\kappa$ B inhibition by COMMD1 are at play, with one possibility being that COMMD1 regulates binding of NF- $\kappa$ B to its target promoter sites. Further research is needed to determine the molecular events resulting in NF- $\kappa$ B inhibition by COMMD1. Some remaining questions include how the interactions among COMMD proteins play a role in this process, as well as whether other effector proteins are required and whether there are posttranslational modifications of players involved in this pathway.

NF- $\kappa$ B has long been recognized as a key transcription factor facilitating the replication of HIV-1 by initiating transcription of the viral genome through  $\kappa$ B sites on the HIV long terminal repeat (Nabel and Baltimore 1987). This link between HIV replication and NF- $\kappa$ B activity led to investigations that revealed that COMMD1 overexpression represses HIV-1 replication in resting CD4<sup>+</sup> lymphocytes, whereas HIV-1 replication is stimulated in these cells by siRNA-mediated knockdown of COMMD1 (Ganesh et al. 2003). These data reveal an unexpected putative role for COMMD1 in protection against HIV infection.

## Concluding Remarks

The cloning of COMMD1 presents the first example of using a purebred dog population from private owners to identify a disease gene. Although no mutations in the *COMMD1* gene have been found to underlie human genetic diseases, its cloning reveals a possible new pathway in copper homeostasis. Further exploration of this pathway may lead to new insights into copper homeostasis and eventually to the identification of new candidate genes for copper-overload disorders of unknown etiology. However, based on the data presented here, COMMD1 appears to be involved in multiple cellular processes (summarized in Figure 3), which is consistent with the ubiquitous expression pattern of COMMD1. Although the pathology of liver cirrhosis, one of the pathological features of canine copper toxicosis, is known to coincide with NF- $\kappa$ B activation, no other signs of an uncontrolled NF- $\kappa$ B signaling pathway nor defects in sodium homeostasis are observed in affected Bedlington terriers.

One might speculate that functional redundancy by other COMMD proteins alleviates the phenotype that one might expect in affected dogs. It will be interesting to investigate if the other COMMD proteins share more functions with COMMD1 than their ability to inhibit NF- $\kappa$ B activation. The exact molecular functions of COMMD1 are unknown, but most probably involve specific COMMD1 protein-protein interactions. A future approach in investigating the functions of COMMD1 will therefore be to continue identifying and characterizing novel protein-protein interactions that involve COMMD1. Other approaches that should prove useful are the generation and characterization of COMMD1-deficient cell lines and knockout mice.

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