Cell. Mol. Life Sci. 64 (2007) 1997 – 2005
1420-682X/07/151997-9
DOI 10.1007/s00018-007-7078-y

Cellular and Molecular Life Sciences

Review

COMMD proteins: COMMing to the scene

G. N. Maine[a, b], E. Burstein[a, b, c, *]

[a]Department of Internal Medicine, Biomedical Science Research Building Rm 1526, University of Michigan Medical School, Ann Arbor, Michigan 48109 (USA); Fax: +1 734 647 7950, e-mail: ezrab@umich.edu
[b]Molecular Mechanisms of Disease Program, Biomedical Science Research Building Rm 1526, University of Michigan Medical School, Ann Arbor, Michigan 48109 (USA)
[c]Gastroenterology Section at the Ann Arbor VA Medical Center, Ann Arbor, Michigan 48105 (USA)

Received 13 February 2007; received after revision 27 March 2007; accepted 24 April 2007
Online First 14 May 2007

Abstract. COMM Domain-containing or COMMD proteins are a recently discovered group of factors defined by the presence of a unique motif in their extreme carboxy termini (Copper metabolism MURR1, or COMM domain). This protein family is comprised of ten members which are widely conserved throughout evolution and share certain functional properties. At the present time, a number of seemingly discrete functions have been ascribed to these factors. These include the regulation of such events as the activity of the transcription factor NF-κB, copper homeostasis, the function of the epithelial sodium channel, and cell proliferation. A unifying mechanism that would explain all these events is lacking at the moment, but recent studies suggest that regulation of the ubiquitin pathway may be the basis of many of the functions of the COMMD protein family.

Keywords. COMMD1, MURR1, COMMD protein, ubiquitin, Cullin, copper, NF-κB, HCaRG, calcium, ENaC.

The *COMMD* gene family

COMM domain-containing or COMMD proteins are a group of evolutionarily conserved factors present in a wide range of multicellular organisms (Fig. 1a) [1]. The defining characteristic of all the members of the family is the presence of a highly conserved and unique domain in the extreme carboxy terminus of these proteins (Fig. 1b).

The founding member of this family, *COMMD1*, was first identified as a gene in close proximity to the imprinted murine gene *U2af1-rs1* [2]. Therefore, it was initially designated as *Murr1* (Mouse U2af1-rs1 region 1). Similarly, an unrelated gene also located in proximity to murine *U2af1-rs1* was named *Murr2*, precluding the use of this root name to designate family members that were discovered a few years later. The genomic organization of the *U2af1-rs1* and *Commd1* loci is unique to the mouse and results in specific transcriptional regulatory events due to the proximity of *Commd1* to this imprinted gene [3, 4]. However, this is not observed in other species, including humans, where the *U2AF1-RS1*gene is located in a different genomic area [2].

Subsequent to its initial designation, the *COMMD1* locus was found to be mutated in canine copper toxicosis and was hence designated as the copper metabolism *MURR1* gene [5]. Additional members of the family were later identified in a biochemical screen for interacting proteins with homology to *MURR1* [1]. This screen co-purified three factors

* Corresponding author.

a

Orthologs in other Species

Conservation (% against human sequence)

	Mammals	Fish	Dictyostelium	Unicellular protozoa
COMMD1	92-95	72-76	58	-
COMMD2	97-99	88	67	50
COMMD3	93	71	53	52
COMMD4	94-97	79	60	50
COMMD5	89-93	70	52	55
COMMD6	93	88	76	-
COMMD7	94-95	81-82	62	47
COMMD8	86-92	70	58	-
COMMD9	93-95	77	68	55
COMMD10	93-99	76-81	56	48

b

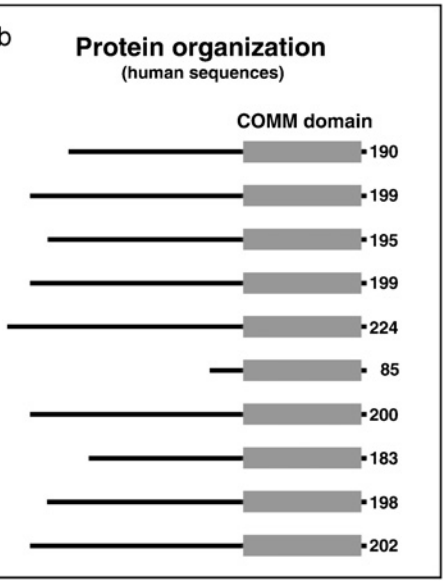

Figure 1. (*a*) Phylogenetic conservation of the *COMMD* gene family. Orthologs for the human *COMMD* genes were identified in various mammalian and fish species and their level of conservation compared against the corresponding human protein (derived from Burstein et al. [1]). In addition, orthologs could be identified in *Dictyostelium discoideum* (accession numbers for the *Dictyostelium* COMMD1 to 10 orthologous proteins are: XP_629812, XP_639903, XP_638867, XP_642944, XP_643905, XP_637929, XP_643770, XP_629572, XP_635194, XP_643748). Finally, *COMMD* orthologs are also present in unicellular protozoa such as *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Tetrahymena thermophila* (accession numbers for the COMMD orthologous proteins identified in unicellular protozoa, namely COMMD2–5, COMMD7, 9 and 10 are: XP_001304865, XP_653799, XP_001319041, XP_001322150, XP_001324444, XP_001007644, XP_001303457). (*b*) Schematic representation of the COMMD family of proteins. The conserved COMM domain is shown in gray, along with the respective amino acid length of each protein in humans.

that had an area of homology with MURR1, which was subsequently designated as the copper metabolism MURR1 domain. Additional searches in NCBI protein databases allowed the identification of all family members, which were subsequently named COMMD1 through 10. The 10 members of this family are found in all vertebrates, with significant conservation observed among mammals and even fish. Additionally, a recent analysis of available sequences indicates that all ten of these factors are also present in *Dictyostelium discoideum*, and several *COMMD* genes are also identifiable in *Drosophila* and *Caenorhabditis* species, as well as in several unicellular protozoa (Fig. 1a).

Most COMMD proteins are about 200 residues in length and all possess a 70 to 85 amino-acids-long carboxy-terminal COMM domain and an α-helical-rich amino terminus (Fig. 1b) [1]. The main exception to this architecture is mammalian COMMD6, which consists primarily of a COMM domain. Outside the COMM domain, each COMMD protein contains a unique amino-terminal region, which are divergent across members of the family but are highly conserved among its orthologs in other species. For example, while human and zebrafish COMMD1 are 72% conserved, human COMMD1 and COMMD10 are only 34% conserved when regions outside the COMM domain are included in the comparison.

The expression of these genes in mammalian tissues has not been extensively studied, and the available data are derived from tissue expression profiling performed at the mRNA level [1]. These data indicate that some *COMMD* genes, like *COMMD1*, are widely expressed in adult tissues. However, other *COMMD* genes demonstrate preferential expression in certain compartments, as in the case of *COMMD9*, which is most expressed in myeloid cells and the central nervous system [6]. However, these data have not been confirmed at the RNA level with another method such as quantitative RT-PCR, nor has the expression of these genes at the protein level been reported to date. Similarly, with the exception of COMMD1 and COMMD5, the cellular localization of these proteins is not known. Cell fractionation and imaging methods demonstrate that COMMD1 is distributed throughout the cell, including the nucleus, as well as in perinuclear organelles that have not been further characterized [1, 7, 8]. COMMD5, on the other hand, is known to be a primarily nuclear protein [9].

The COMM domain not only defines this protein family but provides a critical interface for protein-protein interactions. It has been shown that the

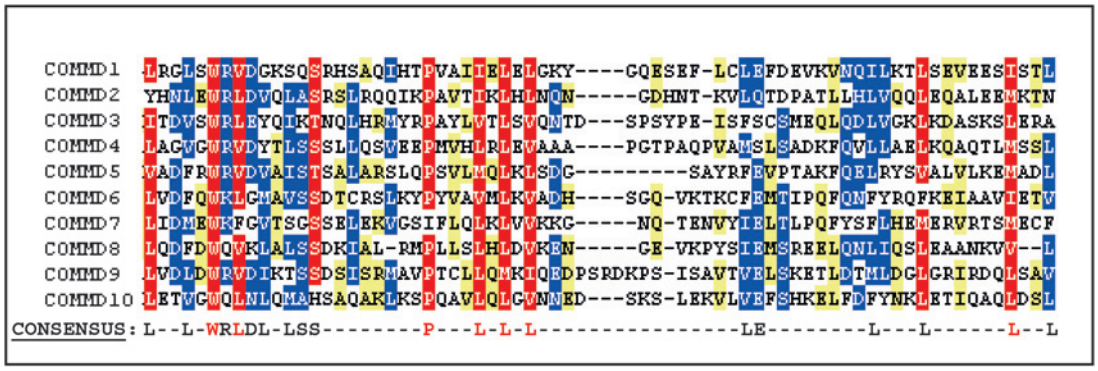

Figure 2. The COMM domain. Alignment of all 10 human COMMD proteins at the level of their respective COMM domains is shown. The degree of conservation of each amino acid residue among these sequences is indicated based on Dayhoff PAM 250 scoring matrices (red, 90% conserved; blue, 70% conserved; yellow, 50% conserved). In addition, the most conserved amino acid residues within the domain are indicated underneath, as determined in an alignment of over 90 homologs in multiple species. Residues marked in red font in the consensus sequence exhibit the highest conservation in the domain when the extended alignment of multiple homologs is analyzed.

COMM domain mediates COMMD1-COMMD1 interactions as well as binding with other COMMD proteins. Interestingly, Narindrasorasak and colleagues recently confirmed the ability of COMMD1 to oligomerize via its COMM domain, and demonstrated that recombinant human COMMD1 primarily migrates as a dimer under non-reducing conditions [10]. Similarly, binding of COMMD1 to other proteins such as Cul2 and it associated factors is mediated by this critical domain [1, 11, 12]. The COMM domain is leucine rich and contains an invariably conserved tryptophan (Fig. 2). The tertiary structure of the COMM domain has not been solved, although secondary-structure algorithms predict the presence of a conserved β sheet and an extreme carboxy-terminal α-helix. On the other hand, the structure of the amino terminus of COMMD1 has been recently reported and demonstrates a packed sequence of five α-helices with the last three adopting a distinct three-helix bundle [13]. Given the growing number of protein-protein interactions that the COMMDs participate in [1, 7, 11, 12, 14–16], additional structural information about these factors, particularly their COMM domain, will be informative in understanding their mechanism of binding.

COMMD proteins regulate NF-κB-mediated transcription

One of the functions first ascribed to COMMD1 (and later to other COMMD proteins) is its ability to inhibit NF-κB [1, 15], a transcription factor that promotes the expression of gene products involved in several processes including cell survival, inflammation, viral replication, and oncogenesis [17–20]. NF-κB consists of dimers formed by conserved proteins that share a defining 300 amino acid sequence termed the Rel Homology Domain (RHD). In mammals, there are five genes that encode members of this family: *RELA, RELB, REL, NFKB1* and *NFKB2*. NF-κB-mediated gene expression is regulated to a large extent by cytoplasmic sequestration of the NF-κB complex. In the canonical NF-κB pathway, this is the result of interactions between the inhibitory IκB proteins and NF-κB complexes [21, 22]. Activation of a multimeric kinase known as the IκB kinase (IKK) complex results in phosphorylation of IκB. Once phosphorylated, IκB is ubiquitinated by a multimeric ubiquitin ligase containing Cul1, targeting it for proteasomal degradation [23, 24]. IκB degradation enables the translocation of NF-κB complexes to the nucleus where they bind to cognate DNA sequences present in an array of promoters, ultimately resulting in induction of gene expression.

The first indication that COMMD1 regulates the NF-κB pathway stemmed from efforts to identify novel interacting partners of the X-linked inhibitor of apoptosis (XIAP) [7]. COMMD1 was identified in a yeast two-hybrid screen as a factor binding to XIAP. This association did not modulate the anti-apoptotic activity of XIAP, but rather abrogated XIAP-mediated activation of NF-κB. As an extension of this observation, COMMD1 was shown to inhibit NF-κB activation mediated by a number of additional stimuli [15]. Subsequent studies identified that to various degrees, all members of the COMMD family were capable of inhibiting NF-κB transcriptional activity, and that the COMM domain plays a critical role in this process [1, 12].

Given the ability of COMMD proteins to broadly inhibit the NF-κB pathway, it can be anticipated that they would be involved in biologic processes that are regulated by NF-κB. One such example is the

replication of HIV-1, a virus that contains two κB-binding sites in its genome and is highly dependent on NF-κB for its replication to occur [25]. Indeed, expression of COMMD1 in $CD4^+$ T cells abrogated HIV-1 replication, whereas endogenous depletion of COMMD1 accelerated it [15]. These observations are consistent with gene expression data in animal models of SIV, in which disease progression correlated well with *COMMD1* levels [26]. Macaques that experienced slow disease progression after SIV infection had higher levels of *COMMD1* mRNA compared to those with typical or accelerated rates of disease progression (GEO database, record GDS172), again suggesting that COMMD1 is a resistance factor against lentiviral infection.

In addition to their effect on viral replication, COMMD proteins negatively regulate expression of a number of pro-inflammatory NF-κB-inducible genes [11]. In response to tumor necrosis factor (TNF) stimulation, cells deficient in COMMD1 accumulate higher amounts of mRNA of κB-dependent genes such as *ICAM1* (290% higher) and *CCL2* (50% higher). Furthermore, conditioned media from these cells promote enhanced chemotaxis of freshly isolated peripheral blood mononuclear cells across a membrane barrier (a 60% increase compared to the control). This increase in chemotaxis rate correlates with an increase in secretion of NF-κB-inducible chemokines by COMMD1-deficient cells, such as CCL2 (by 56% compared to the control). These data collectively demonstrate that by inhibiting NF-κB activity, COMMD1 regulates HIV-1 replication, pro-inflammatory gene expression, and possibly other processes that remain to be elucidated.

The ability of COMMD proteins to inhibit NF-κB activation in response to a broad array of stimuli suggested that they function downstream of the IKK complex, a multimeric kinase required for NF-κB activation. COMMD1 does not interact with IKK, but rather associates with NF-κB subunits themselves, a property shared by other members of the COMMD family [1, 15]. Specifically, COMMD proteins interact with a motif present in the conserved RHD of NF-κB. The IκB family of inhibitory proteins also associate with NF-κB, and this interaction inhibits nuclear accumulation of NF-κB by masking the nuclear localization signal present in the RHD [27]. However, the binding of COMMD1 with RelA, the most abundant NF-κB subunit, does not affect nuclear translocation, a finding consistent with the fact that COMMD1 and IκB-α interact with different motifs within the RHD.

These findings indicate that COMMD1-mediated inhibition of NF-κB occurs through a mechanism distinct from the effects of IκB proteins. Other pathways of transcriptional suppression have been described and involve the control of the activity of NF-κB in the nucleus [28–31]. Indeed, COMMD1 was shown to negatively regulate the association of nuclear NF-κB with chromatin [1]. Endogenous depletion of COMMD1 results in more sustained recruitment of RelA to κB-dependent promoter elements, whereas expression of COMMD1 decreases the duration of time RelA remains bound to chromatin. Interestingly, COMMD1 itself was recruited to DNA in a stimulus-dependent manner, although the mechanism for this recruitment has not been further elucidated. These data indicate that COMMD proteins terminate NF-κB-mediated transcriptional responses by destabilizing the interaction between NF-κB and its binding sites on chromatin. The mechanism by which this process occurs was poorly understood until recently.

COMMD1 regulation of NF-κB through a Cullin-containing ubiquitin ligase

Work by Saccani and colleagues demonstrated that in addition to IκB-dependent pathways, termination of NF-κB-mediated gene expression requires ubiquitination of DNA-bound RelA, which targets it for proteasomal degradation [29] (Fig. 3). This raised the possibility that the ability of COMMD1 to affect RelA-chromatin interactions was connected to the ubiquitin-proteasome pathway. Various data indicate that this is the case, as expression of COMMD1 in cells promotes accumulation of endogenous ubiquitinated RelA, whereas endogenous depletion of COMMD1 diminishes the pool of ubiquitinated RelA [11]. These observations correlate with the finding that cells deficient in COMMD1 express higher protein levels of endogenous NF-κB subunits, namely RelA, RelB, p100 and p105. Importantly, endogenous COMMD1 immunoprecipitated complexes can catalyze the formation of poly-ubiquitin chains in an *in vitro* ubiquitination reaction, indicating that COMMD1 associates with an E3 ubiquitin ligase. The identity of this ligase and its involvement in NF-κB ubiquitination was subsequently determined.

Work by Ryo et al. [32] demonstrated that SOCS1, a member of the SOCS family of proteins that share a conserved motif in their carboxy termini called the SOCS box, is capable of ubiquitinating RelA [32]. SOCS1 is also the substrate recognition component of a multimeric ubiquitin ligase containing a Cullin protein [33, 34]. These complexes are referred to as ECS and contain elongins B/C, Cullin 2 or 5, and a SOCS box-containing protein. Therefore, the possibility that COMMD1 might facilitate the ubiquitina-

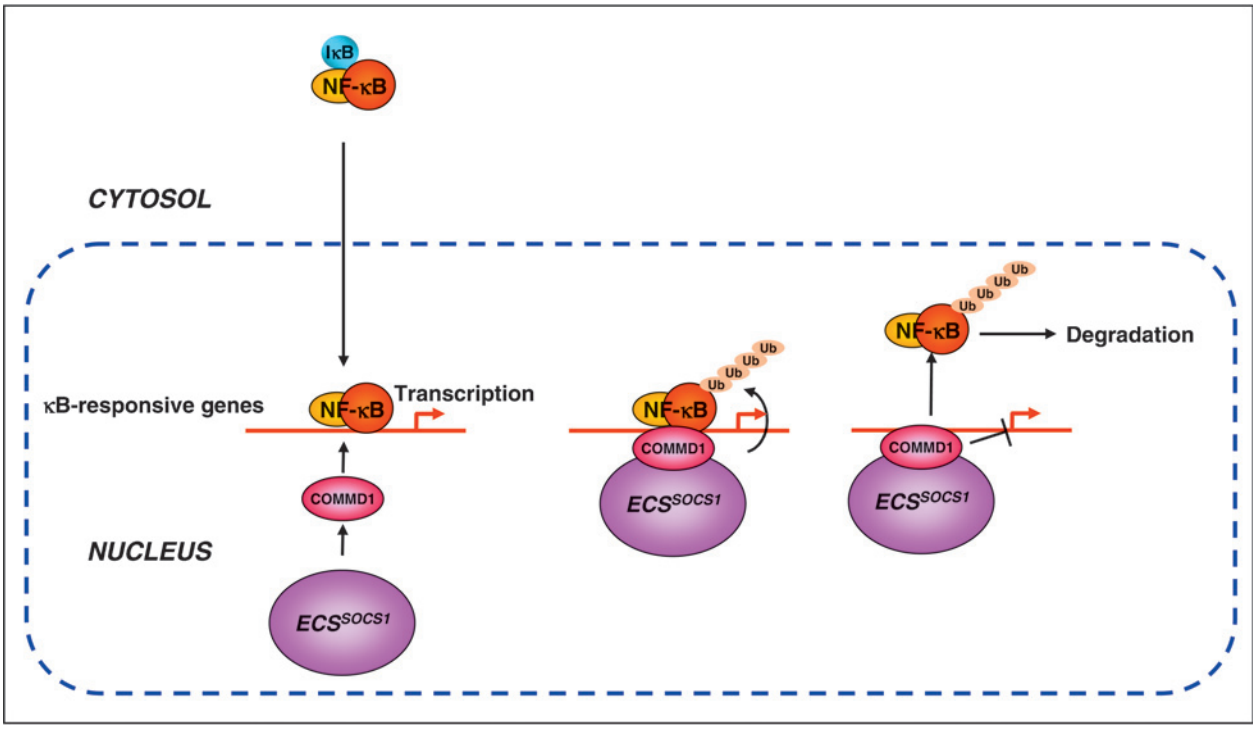

Figure 3. Model of COMMD1-mediated negative regulation of NF-κB. Activation of the NF-κB pathway enables translocation of NF-κB dimers to the nucleus where they bind cognate promoter elements to induce gene expression. COMMD1 is recruited to chromatin, and later becomes associated with the ECS^{SOCS1} ubiquitin ligase. We speculate that this results in the recruitment of the entire ubiquitin ligase complex to these promoter sites, facilitating poly-ubiquitination of NF-κB subunits. This event ultimately targets NF-κB for proteasomal degradation and termination of transcription.

tion of NF-κB through the ECS^{SOCS1} complex was examined [11]. Indeed, precipitation of COMMD1 from cells was able to recover SOCS1 and other components of the ubiquitin ligase. Furthermore, COMMD1 immunoprecipitates from cells expressing ECS^{SOCS1} catalyzed poly-ubiquitination of recombinant RelA in an *in vitro* ubiquitination reaction. Finally, depletion of endogenous Cul2 or SOCS1 by RNA interference abrogated the ability of COMMD1 to promote *in vivo* ubiquitination of RelA. These data demonstrate that COMMD1 exerts its inhibitory activity by associating with the ECS^{SOCS1} ubiquitin ligase, resulting in ubiquitination and proteasomal degradation of NF-κB subunits. Interestingly, the recruitment of COMMD1 to promoter sites and its ability to regulate NF-κB-chromatin interactions suggests that these events are likely orchestrated locally on chromatin (Fig. 3).

Studies to ascertain the mechanism of binding between COMMD1 and the ECS^{SOCS1} complex revealed that COMMD1 binds independently to the Cul2 and SOCS1 subunits of the ligase [11]. The interaction of COMMD1 with Cul2 was mapped to a region that contains the conserved Cullin homology domain. Consistent with this, COMMD1 is also capable of interacting with other Cullin proteins, suggesting that COMMD1 may associate with other ubiquitin ligases. The binding between COMMD1 and Cul2 is inducible by TNF stimulation, indicating that the composition of the complex is affected by activation of the NF-κB pathway. Independent of Cul2, COMMD1 also associates with the SH2 domain of SOCS1 in a manner not affected by TNF. SH2 domains typically provide a binding platform for proteins containing phosphorylated tyrosine residues, but it remains to be determined if such a modification of COMMD1 is required for its association with SOCS1. The functional significance of this interaction is that it enhances recruitment of the ubiquitin ligase to its NF-κB substrate, as COMMD1 stabilizes the binding between SOCS1 and the amino terminus of RelA. These findings identify a novel architecture for Cullin-containing ubiquitin ligases, and suggest the possibility of broader roles for COMMD1 and its homologs in biological processes through the regulation of the ubiquitination pathway.

COMMD1 as a regulator of copper metabolism

Copper is an essential trace element that acts as a catalytic co-factor for a number of enzymes. However,

free copper is highly toxic due to its ability to generate hydroxyl radicals, and eukaryotes therefore have delicate and highly conserved mechanisms to handle this transition metal [35]. Mutations in the genes involved in copper homeostasis predictably result in human disease [36]. Studies to identify susceptibility genes associated with a copper toxicosis disorder observed in a purebred dog population led to the identification of a mutation in the *COMMD1* locus in affected animals, implicating this gene in copper metabolism [5].

Hardy and colleagues originally described that Bedlington terriers have a high incidence of a unique copper toxicosis disorder, inherited in an autosomal recessive manner [37]. Canine copper toxicosis is characterized by a defect in biliary excretion of copper, resulting in the progressive accumulation of copper in the liver leading to chronic hepatitis and cirrhosis [38]. In humans, the main form of copper toxicosis is Wilson disease, which is caused by mutations in the gene encoding the hepatic copper transporter *ATP7B* [36]. While copper toxicosis in Bedlington terriers resembles Wilson disease, the canine ortholog of *ATP7B* and other known copper regulators are not mutated in affected dogs [39]. This prompted the search for the causative gene, which was presumed to be a novel regulator of copper metabolism.

Positional cloning efforts determined that an ~ 10 kb deletion involving exon 2 of the *COMMD1* gene is associated with the disease phenotype in European pedigrees [5], and subsequent studies in Australia have observed that all dogs homozygous for this deletion develop the disorder [40]. Consistent with a disease-causing role for this *COMMD1* mutation, the protein is absent in affected animals that are homozygous for the exon 2 deletion [8]. Moreover, reduction of *COMMD1* expression by RNA interference results in increased accumulation of copper in human embryonic kidney cells [7] and in dog hepatic cells [41], supporting the notion that *COMMD1* plays a role in the control of intracellular copper levels.

More recently, it has been demonstrated that not all affected Bedlington terriers carry a deletion in exon 2 of the *COMMD1* gene; some affected animals are heterozygous for the *COMMD1*-deleted state or even carry two alleles without this deletion [40, 42]. To date, no disease-causing mutations have been found in the *COMMD1* coding sequence or splice sites of non-deleted alleles that segregate with the disorder, raising questions about the nature of the mutation that results in copper toxicosis in these animals. Importantly, *COMMD1* mRNA or protein levels in these dogs have not been reported, leaving open the possibility that there is still another mutation in this gene, for example in the promoter region, that compromises the expression of *COMMD1*.

Following the description of mutations in the *COMMD1* gene of dogs affected with copper toxicosis, similar analyses have been performed in humans [43–47]. Several reports examining patients affected with non-Wilsonian copper toxicosis, or patients with Wilson disease (including cohorts with atypical features) have not identified disease – causing genetic changes in the *COMMD1* gene. Therefore, human examples of a disease caused by mutations in the *COMMD1* locus remain to be identified. An additional area of uncertainty at the moment is whether other *COMMD* genes play a similar role in intracellular copper regulation, a possibility that is plausible based on sequence and functional homologies between these factors.

The mechanism by which COMMD1 controls copper excretion has only been partially elucidated. COMMD1 interacts with the copper transporter ATP7B, which is responsible for biliary copper excretion [16]. The interaction between these molecules was mapped to the amino terminus of ATP7B, a region containing the metal-binding domains of the molecule. It has been inferred from this finding that COMMD1 somehow facilitates ATP7B-mediated copper excretion, although the mechanism for this effect is still unknown. Recently, recombinant human COMMD1 was shown to bind copper *in vitro*, although the role of this association *in vivo* remains to be established [10]. Interestingly, ATP7B deficiency results in loss of biliary excretion of copper as well as lack of copper incorporation into secreted proteins produced in the liver, with ceruloplasmin being the most notable example [36]. However, unlike patients with Wilson disease, COMMD1-deficient Bedlington terriers have only small changes in ceruloplasmin levels [38]. This implies that the defect in copper handling is specific to its biliary excretion and that other functions of ATP7B, such as facilitating the incorporation of copper into ceruloplasmin in COMMD1-deficient animals, are largely preserved. Therefore, these data predict that the regulatory effect played by COMMD1 in this pathway is very specific.

COMMD1-mediated control of copper levels is regulated by XIAP [7]. The connection between XIAP, COMMD1, and copper was first established when XIAP was found to interact with COMMD1 in a yeast two-hybrid screen. XIAP affects intracellular copper levels, but in contrast to COMMD1, XIAP mediates copper accumulation. Additional studies demonstrated that XIAP, utilizing its carboxy-terminal RING domain, serves as an E3 ubiquitin ligase for

COMMD1, thereby facilitating its degradation. The aggregate of the data indicate that it is through this regulation of COMMD1 that XIAP participates in copper homeostasis. More recently, XIAP was found to be a copper-binding protein, whose levels are under the influence of intracellular copper stores, possibly providing for negative feedback via COMMD1 [48].

Other reported functions

In addition to their involvement in the NF-κB pathway and copper homeostasis, COMMD proteins have been reported to play a role in the regulation of other functions. In an effort to identify genes associated with abnormal calcium homeostasis, Johanne Tremblay's group cloned and characterized a gene that has higher expression in the parathyroid gland and kidney of spontaneously hypertensive rats, which was named hypertension-related, calcium-regulated gene or *HCaRG* [9,49]. This gene was subsequently identified as a member of the *COMMD* gene family and renamed *COMMD5* by the HUGO gene nomenclature committee. COMMD5 is predominantly nuclear and its expression is affected by extracellular calcium levels. Furthermore, *COMMD5* appears to be developmentally regulated, as gene expression is more prominent in adult tissues, compared to those from fetal organs. Functional studies indicated that expression of *COMMD5* in cells impaired their proliferative capacity and induced a more differentiated phenotype, which correlated with an increase in the expression of the cyclin-dependent kinase (CDK) inhibitor, $p21^{Cip1/WAF1}$ [50]. Flow-cytometric analysis of DNA content as a measure of cell cycle progression demonstrated that cells expressing *COMMD5* preferentially exhibit G2/M arrest, consistent with an increase in $p21^{Cip1/WAF1}$. Interestingly, *COMMD5* levels were decreased in tumors compared to normal tissues, raising the possibility that this gene might be involved in cell cycle regulation in human cancer.

In addition to promoting cell cycle arrest, *COMMD5* overexpression results in differentiation changes including the formation of lamellipodia, structures involved in cell motility [51]. This is consistent with the increased rate of migration demonstrated by COMMD5-expressing cells. The increase in migratory capacity correlated with heightened expression of genes involved in lamellipodia formation, including transforming growth factor-α (TGF-α). Although the mechanism by which COMMD5 affects TGF-α production is still unknown, the phenotypic changes observed in cells expressing COMMD5 seem to be mediated by an autocrine TGF-α loop.

COMMD1 has also been implicated in sodium homeostasis. Biasio and colleagues demonstrated that COMMD1 interacts with the human epithelial sodium (Na) channel (ENaC), an observation gathered from a yeast two-hybrid screen utilizing the δ subunit of ENaC as bait [14]. Further binding studies utilizing recombinant proteins or overexpression in cells demonstrated that COMMD1 can bind to the carboxy terminus of the δ subunit, as well as to other ENaC subunits, namely β and γ, but not α. Functionally, expression of COMMD1 impairs the sodium current through ENaC channels in *Xenopus* oocytes. However, the mechanism by which COMMD1 regulates ENaC function remains unknown. Interestingly, the α, β, and γ subunits of ENaC are negatively regulated by ubiquitination mediated by Nedd4, a HECT domain-containing ubiquitin ligase [52]. The regulation by Nedd4 requires a conserved PPxY motif in the carboxy terminus of ENaC subunits, which is not present in the δ subunit. Whether ubiquitination is a mechanism for regulating δENaC is not known; however, its regulation by COMMD1 raises the possibility that COMMD1 may mediate its effects via a ubiquitin-proteasome pathway, similar to its role in the termination of NF-κB responses.

Conclusions

COMMD proteins represent a set of regulatory factors involved in several biological processes important for cellular homeostasis. These include the regulation of copper and sodium transport, NF-κB activity, cell cycle progression, and others that have yet to be determined. The importance of COMMD proteins in cellular homeostasis is further illustrated by the observation that targeted deletion of several members of this family result in embryonic lethality in mice [E. Burstein, unpublished observations]. One possible unifying mechanism of action for the regulatory properties of the COMMD protein family might be their involvement in the ubiquitin-proteasome pathway. As was recently described, COMMD1 facilitates the termination of the NF-κB response through its association with a Cul2-containing ubiquitin ligase (Fig. 3). Given that COMMD1 can also interact with other Cullin proteins, we predict that COMMD proteins likely associate with other ubiquitin ligases. We suspect that the characterization of these enzymes and their relevant substrates will prove to be important in uncovering additional pathways that are controlled by COMMD proteins.

1 Burstein, E., Hoberg, J. E., Wilkinson, A. S., Rumble, J. M., Csomos, R. A., Komarck, C. M., Maine, G. N., Wilkinson, J. C., Mayo, M. W. and Duckett, C. S. (2005). COMMD proteins: a novel family of structural and functional homologs of MURR1. J. Biol. Chem. 280, 22222 – 22232.

2 Nabetani, A., Hatada, I., Morisaki, H., Oshimura, M. and Mukai, T. (1997). Mouse *U2af1-rs1* is a neomorphic imprinted gene. Mol. Cell. Biol. 17, 789 – 798.

3 Wang, Y., Joh, K., Masuko, S., Yatsuki, H., Soejima, H., Nabetani, A., Beechey, C. V., Okinami, S. and Mukai, T. (2004). The mouse Murr1 gene is imprinted in the adult brain, presumably due to transcriptional interference by the antisense-oriented U2af1-rs1 gene. Mol. Cell. Biol. 24, 270 – 279.

4 Zhang, Z., Joh, K., Yatsuki, H., Wang, Y., Arai, Y., Soejima, H., Higashimoto, K., Iwasaka, T. and Mukai, T. (2006). Comparative analyses of genomic imprinting and CpG island-methylation in mouse Murr1 and human MURR1 loci revealed a putative imprinting control region in mice. Gene 366, 77 – 86.

5 van de Sluis, B., Rothuizen, J., Pearson, P.L., van Oost, B.A. and Wijmenga, C. (2002). Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum. Mol. Genet. 11, 165 – 173.

6 Su, A.I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K. A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G., Cooke, M. P., Walker, J. R. and Hogenesch, J. B. (2004). A gene atlas of the mouse and human protein-encoding transcriptomes. Proc. Natl. Acad. Sci. USA 101, 6062 – 6067.

7 Burstein, E., Ganesh, L., Dick, R. D., van De Sluis, B., Wilkinson, J. C., Klomp, L. W., Wijmenga, C., Brewer, G. J., Nabel, G. J. and Duckett, C. S. (2004). A novel role for XIAP in copper homeostasis through regulation of MURR1. EMBO J 23, 244 – 254.

8 Klomp, A.E., van de Sluis, B., Klomp, L.W. and Wijmenga, C. (2003). The ubiquitously expressed MURR1 protein is absent in canine copper toxicosis. J. Hepatol. 39, 703 – 709.

9 Solban, N., Jia, H. P., Richard, S., Tremblay, S., Devlin, A. M., Peng, J., Gossard, F., Guo, D. F., Morel, G., Hamet, P., Lewanczuk, R. and Tremblay, J. (2000). HCaRG, a novel calcium-regulated gene coding for a nuclear protein, is potentially involved in the regulation of cell proliferation. J. Biol. Chem. 275, 32234 – 32243.

10 Narindrasorasak, S., Kulkarni, P., Deschamps, P., She, Y.M. and Sarkar, B. (2007). Characterization and copper binding properties of human COMMD1 (MURR1). Biochemistry 46, 3116 – 3128.

11 Maine, G.N., Mao, X., Komarck, C.M. and Burstein, E. (2007). COMMD1 promotes the ubiquitination of NF-κB subunits through a Cullin-containing ubiquitin ligase. EMBO J. 26, 436 – 447.

12 de Bie, P., van de Sluis, B., Burstein, E., Duran, K.J., Berger, R., Duckett, C.S., Wijmenga, C. and Klomp, L.W. (2006). Characterization of COMMD protein-protein interactions in NF-κB signalling. Biochem. J. 398, 63 – 71.

13 Sommerhalter, M., Zhang, Y. and Rosenzweig, A.C. (2007). Solution structure of the COMMD1 N-terminal domain. J. Mol. Biol. 365, 715 – 721.

14 Biasio, W., Chang, T., McIntosh, C.J. and McDonald, F.J. (2004). Identification of Murr1 as a regulator of the human δ epithelial sodium channel. J. Biol. Chem. 279, 5429 – 5434.

15 Ganesh, L., Burstein, E., Guha-Niyogi, A., Louder, M. K., Mascola, J. R., Klomp, L. W., Wijmenga, C., Duckett, C. S. and Nabel, G. J. (2003). The gene product Murr1 restricts HIV-1 replication in resting $CD4^+$ lymphocytes. Nature 426, 853 – 857.

16 Tao, T.Y., Liu, F., Klomp, L., Wijmenga, C. and Gitlin, J.D. (2003). The copper toxicosis gene product Murr1 directly interacts with the Wilson disease protein. J. Biol. Chem. 278, 41593 – 41596.

17 Green, D.R. (2003). Death and NF-κB in T cell activation: life at the edge. Mol. Cell 11, 551 – 552.

18 Karin, M. and Lin, A. (2002). NF-κB at the crossroads of life and death. Nat Immunol. 3, 221 – 227.

19 Perkins, N.D. (2000). The Rel/NF-κB family: friend and foe. Trends Biochem. Sci. 25, 434 – 440.

20 Silverman, N. and Maniatis, T. (2001). NF-κB signaling pathways in mammalian and insect innate immunity. Genes Dev. 15, 2321 – 2342.

21 Chen, Z., Hagler, J., Palombella, V.J., Melandri, F., Scherer, D., Ballard, D. and Maniatis, T. (1995). Signal-induced site-specific phosphorylation targets IκBα to the ubiquitin-proteasome pathway. Genes Dev. 9, 1586 – 1597.

22 Henkel, T., Machleidt, T., Alkalay, I., Krönke, M., Ben-Neriah, Y. and Baeuerle, P.A. (1993). Rapid proteolysis of IκB-α is necessary for activation of transcription factor NF-κB. Nature 365, 182 – 185.

23 Li, Z.W., Chu, W., Hu, Y., Delhase, M., Deerinck, T., Ellisman, M., Johnson, R. and Karin, M. (1999). The IKKβ subunit of IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis. J. Exp. Med. 189, 1839 – 1845.

24 Sizemore, N., Lerner, N., Dombrowski, N., Sakurai, H. and Stark, G.R. (2002). Distinct roles of the IκB kinase α and β subunits in liberating nuclear factor κB (NF-κB) from IκB and in phosphorylating the p65 subunit of NF-κB. J. Biol. Chem. 277, 3863 – 3869.

25 Hiscott, J., Kwon, H. and Genin, P. (2001). Hostile takeovers: viral appropriation of the NF-kB pathway. J. Clin. Invest. 107, 143 – 151.

26 Vahey, M.T., Nau, M.E., Taubman, M., Yalley-Ogunro, J., Silvera, P. and Lewis, M.G. (2003). Patterns of gene expression in peripheral blood mononuclear cells of rhesus macaques infected with SIVmac251 and exhibiting differential rates of disease progression. AIDS Res Hum Retroviruses 19, 369 – 387.

27 Malek, S., Huxford, T. and Ghosh, G. (1998). IκBα functions through direct contacts with the nuclear localization signals and the DNA binding sequences of NF-κB. J. Biol. Chem. 273, 25427 – 25435.

28 Chen, L., Fischle, W., Verdin, E. and Greene, W.C. (2001). Duration of nuclear NF-κB action regulated by reversible acetylation. Science 293, 1653 – 1657.

29 Saccani, S., Marazzi, I., Beg, A.A. and Natoli, G. (2004). Degradation of promoter-bound p65/RelA is essential for the prompt termination of the nuclear factor κB response. J. Exp. Med. 200, 107 – 113.

30 Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A. and Mayo, M.W. (2004). Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. 23, 2369 – 2380.

31 Hoberg, J.E., Yeung, F. and Mayo, M.W. (2004). SMRT derepression by the IκB kinase α: a prerequisite to NF-κB transcription and survival. Mol. Cell 16, 245 – 255.

32 Ryo, A., Suizu, F., Yoshida, Y., Perrem, K., Liou, Y. C., Wulf, G., Rottapel, R., Yamaoka, S. and Lu, K. P. (2003). Regulation of NF-κB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. Mol. Cell 12, 1413 – 1426.

33 Petroski, M.D. and Deshaies, R.J. (2005). Function and regulation of Cullin-ring ubiquitin ligases. Mol. Cell. Biol. 6, 9 – 20.

34 Willems, A.R., Schwab, M. and Tyers, M. (2004). A hitchhiker's guide to the Cullin ubiquitin ligases: SCF and its kin. Biochim. Biophys. Acta 1695, 133 – 170.

35 Puig, S. and Thiele, D.J. (2002). Molecular mechanisms of copper uptake and distribution. Curr. Opin. Chem. Biol. 6, 171 – 180.

36 Mercer, J.F. (2001). The molecular basis of copper-transport diseases. Trends Mol. Med. 7, 64 – 69.

37 Ludwig, J., Owen, C.A., Jr., Barham, S.S., McCall, J.T. and Hardy, R.M. (1980). The liver in the inherited copper disease of Bedlington terriers. Lab. Invest. 43, 82 – 87.

38 Su, L.C., Ravanshad, S., Owen, C.A., Jr., McCall, J.T., Zollman, P.E. and Hardy, R.M. (1982). A comparison of copper-loading disease in Bedlington terriers and Wilson's disease in humans. Am. J. Physiol. 243, G226-G230.

39 Dagenais, S.L., Guevara-Fujita, M., Loechel, R., Burgess, A.C., Miller, D.E., Yuzbasiyan-Gurkan, V., Brewer, G.J. and Glover, T.W. (1999). The canine copper toxicosis locus is not syntenic with ATP7B or ATX1 and maps to a region showing homology to human 2p21. Mamm. Genome 10, 753 – 756.
40 Hyun, C., Lavulo, L.T. and Filippich, L.J. (2004). Evaluation of haplotypes associated with copper toxicosis in Bedlington terriers in Australia. Am. J. Vet. Res. 65, 1573 – 1579.
41 Spee, B., Arends, B., van Wees, A.M., Bode, P., Penning, L.C. and Rothuizen, J. (in press). Functional consequences of RNA interference targeting COMMD1 in a canine hepatic cell line in relation to copper toxicosis. Anim. Genet..
42 Coronado, V.A., Damaraju, D., Kohijoki, R. and Cox, D.W. (2003). New haplotypes in the Bedlington terrier indicate complexity in copper toxicosis. Mamm Genome 14, 483 – 491.
43 Stuehler, B., Reichert, J., Stremmel, W. and Schaefer, M. (2004). Analysis of the human homologue of the canine copper toxicosis gene MURR1 in Wilson disease patients. J. Mol. Med. 82, 629 – 634.
44 Weiss, K.H., Merle, U., Schaefer, M., Ferenci, P., Fullekrug, J. and Stremmel, W. (2006). Copper toxicosis gene MURR1 is not changed in Wilson disease patients with normal blood ceruloplasmin levels. World J. Gastroenterol. 12, 2239 – 2242.
45 Lovicu, M., Dessi, V., Lepori, M. B., Zappu, A., Zancan, L., Giacchino, R., Marazzi, M. G., Iorio, R., Vegnente, A., Vajro, P., Maggiore, G., Marcellini, M., Barbera, C., Kostic, V., Farci, A. M., Solinas, A., Altuntas, B., Yuce, A., Kocak, N., Tsezou, A., De Virgiliis, S., Cao, A. and Loudianos, G. (2006). The canine copper toxicosis gene MURR1 is not implicated in the pathogenesis of Wilson disease. J. Gastroenterol. 41, 582 – 587.
46 Wu, Z.Y., Zhao, G.X., Chen, W.J., Wang, N., Wan, B., Lin, M.T., Murong, S.X. and Yu, L. (2006). Mutation analysis of 218 Chinese patients with Wilson disease revealed no correlation between the canine copper toxicosis gene MURR1 and Wilson disease. J. Mol. Med. 84, 438 – 442.
47 Coronado, V.A., Bonneville, J.A., Nazer, H., Roberts, E.A. and Cox, D.W. (2005). COMMD1 (MURR1) as a candidate in patients with copper storage disease of undefined etiology. Clin. Genet. 68, 548 – 551.
48 Mufti, A.R., Burstein, E., Csomos, R. A., Graf, P. C., Wilkinson, J. C., Dick, R. D., Challa, M., Son, J. K., , Bratton, S. B., Su, G. L., Brewer, G. J., Jakob, U. and Duckett, C. S. (2006). XIAP is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. Mol. Cell. 21, 775 – 785.
49 Solban, N., Dumas, P., Gossard, F., Sun, Y., Pravenec, M., Kren, V., Lewanczuk, R., Hamet, P. and Tremblay, J. (2002). Chromosomal mapping of HCaRG, a novel hypertension-related, calcium-regulated gene. Folia Biol. (Praha) 48, 9 – 14.
50 Devlin, A.M., Solban, N., Tremblay, S., Gutkowska, J., Schurch, W., Orlov, S. N., Lewanczuk, R., Hamet, P. and Tremblay, J. (2003). HCaRG is a novel regulator of renal epithelial cell growth and differentiation causing G2M arrest. Am. J. Physiol. Renal Physiol. 284, F753-F762.
51 El Hader, C., Tremblay, S., Solban, N., Gigras, D., Beliveau, R., Orlov, S.N., Hamet, P. and Tremblay, J. (2005). HCaRG increases renal cell migration by a TGF-α autocrine loop mechanism. Am. J. Physiol. Renal Physiol. 289, F1273-F1280.
52 Malik, B., Price, S.R., Mitch, W.E., Yue, Q. and Eaton, D.C. (2006). Regulation of epithelial sodium channels by the ubiquitin-proteasome proteolytic pathway. Am. J. Physiol. Renal Physiol. 290, F1285 – F1294.