CBS domains: structure, function, and pathology in human proteins

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CBS domains: structure, function, and pathology in human proteins. Am J Physiol Cell Physiol 289: C1369–C1378, 2005; doi:10.1152/ajpcell.00282.2005.—The cystathionine-β-synthase (CBS) domain is an evolutionarily conserved protein domain that is present in the proteome of archaea, bacteria, and eukarya. CBS domains usually come in tandem repeats, which are found in cytosolic and membrane proteins performing different functions (metabolic enzymes, kinases, and channels). Crystallographic studies of bacterial CBS domains have shown that two CBS domains form an intramolecular dimeric structure (CBS pair). Several human hereditary diseases (homocystinuria, retinitis pigmentosa, hypertrrophic cardiomyopathy, myotonia congenital, etc.) can be caused by mutations in CBS domains of, respectively, cystathionine-β-synthase, inosine 5′-monophosphate dehydrogenase, AMP kinase, and chloride channels. Despite their clinical relevance, it remains to be established what the precise function of CBS domains is and how they affect the structural and/or functional properties of an enzyme, kinase, or channel. Depending on the protein in which they occur, CBS domains have been proposed to affect multimerization and sorting of proteins, channel gating, and ligand binding. However, recent experiments revealing that CBS domains can bind adenosine-containing ligands such ATP, AMP, or S-adenosylmethionine have led to the hypothesis that CBS domains function as sensors of intracellular metabolites.

PROTEIN DOMAINS are evolutionarily conserved units with shared structural (sequence and folding pattern) and functional properties. It is generally assumed that each protein domain performs a specific function. However, because an identical domain may occur in proteins with completely different functions (e.g., channels, kinases, metabolic enzyme), a major challenge is to relate the domain function to the overall protein function. A nice example of this conundrum is posed by cystathionine-β-synthase (CBS) domains. These domains were first described in 1997 by Bateman (3), who identified in the genome of the archaebacterium Methanococcus jannaschii a group of proteins containing one or more copies of a conserved domain of ~60 amino acids. Homology screening of protein databases revealed that this domain was widespread, not only in archaebacterial proteins but also in eubacterial and eukaryotic proteins, e.g., human CBS, from which the name of the domain is derived (3). Moreover, this analysis also indicated that CBS domains usually come in tandem repeats, which associate to form a so-called Bateman domain or a CBS pair. An up-to-date list of proteins with CBS domains is found in the NCBI Conserved Domain Database (see http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=pfam00571). Table 1 summarizes the occurrence of CBS domain-containing proteins in prokarya and eukarya.

Although their precise function remains to be elucidated, the (patho)physiological importance of CBS domains is emphasized by the observation that point mutations in CBS domains can seriously cripple the specific protein function and are responsible for several hereditary diseases in humans (see also Fig. 5): retinitis pigmentosa [RP; mutation in inosine-5′-monophosphate dehydrogenase (IMPDH)] (38), homocystinuria (CBS) (69), familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome [AMP-activated protein kinase (AMPK)] (4, 22, 23), myotonia congenital (CIC-1) (64), idiopathic generalized epilepsy (CIC-2) (28), Dent’s disease (CIC-5) (46), osteopetrosis (CIC-7) (9, 42), and Bartter syndrome (CIC-Kb) (41). In this review, we first focus on the structure of CBS domains and then discuss the role of these domains in the function or dysfunction of IMPDH, CBS, AMPK, and CLC chloride channels.

STRUCTURE OF CBS DOMAINS

The first clue as to the structure of CBS domains came from the crystal structure of Chinese hamster (Cricetulus griseus) IMPDH, which contains two CBS domains in tandem (71). On the basis of these data, it was proposed that a single CBS domain consists of a conserved β1-α1-β2-α2 pattern (see Fig. 1). However, the CBS domains appeared relatively disordered in the crystal structure, which precluded a detailed structural view of the CBS pair, i.e., the overall structure formed by the association of the two domains. More recently, the crystal structures of several bacterial proteins containing one or more CBS domains have been resolved, e.g., IMPDH from Streptococcus pyogenes (Protein Data Bank code 1ZFJ) (78) and three functionally uncharacterized proteins from, respectively, Thermotoga maritima (PDB code 1OS0) (53), Thermoplasma acidophilum (PDB code 1PVM) (see http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics&pdbId=1PVM&page), and Methanothermobacter thermautotrophicus (PDB code 1BQJ) (see http://www.rcsb.org/pdb/cgi/explore.

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cgi?job=graphics&pdbId=1PBJ&page). These data confirm the originally proposed pattern for a single CBS domain, albeit with a cautionary note for the first -strand (β1), which is less reliably predicted in some structures (see Fig. 2A). Moreover, these crystal structures also show how two CBS domains associate to form a CBS pair (Fig. 2B). In essence, a CBS pair is a symmetrical structure that is composed of two (β1)-α1-β2-β3-α2 units in an antiparallel arrangement. The four α-helices are positioned at one side of the pair (Fig.

Table 1. Current distribution of CBS domains among different phyla

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Proteins With CBS Domains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>0</td>
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<tr>
<td>Prokarya</td>
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<tr>
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<td>Eubacteria</td>
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<tr>
<td>Chordata</td>
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</tr>
<tr>
<td>Homo sapiens</td>
<td>50</td>
</tr>
<tr>
<td>Arthropoda</td>
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<td>Drosophila melanogaster</td>
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</tr>
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<td>Nematoda</td>
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<tr>
<td>Caenorhabditis elegans</td>
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<tr>
<td>Fungi</td>
<td>104</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>12</td>
</tr>
</tbody>
</table>

CBS, cystathione β-synthase. Data were drawn from http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR000644.
CBS domains can adopt the typical \((\beta_1)_{\alpha_1}\beta_2\beta_3\alpha_2\) core structure and form an intramolecular CBS pair.

It should be noted that in the bacterial proteins the CBS pairs are always formed by intramolecular association of two CBS domains. Although at this time one cannot formally exclude the formation of an intermolecular CBS pair, the presently available data indicate that the above-described interaction between two CBS domains (being limited to intramolecular domains) is not a structural determinant for protein multimerization. A second conclusion that can be drawn from the available structures of CBS-containing enzymes is that CBS domains are not part of the catalytic center. In \(S. pyogenes\) IMPDH, the CBS domains are at the periphery of the enzyme complex and outside the catalytic center (78). Similarly, the truncated human CBS, lacking the CBS domains, is still catalytically active, although the regulation of the enzyme activity is disturbed (50).

**HUMAN CBS PROTEINS AND ASSOCIATED DISEASES**

CBS domains have been identified in a wide range of proteins, both soluble and membrane proteins. CBS domain-containing proteins have very divergent functions, ranging from metabolic enzymes and transcriptional regulators to ion channels and transporters. Therefore, an intriguing question is whether CBS domains serve a common function in these different proteins or whether CBS domains are polyfunctional units. To discuss this problem, we give an overview of human (mammalian) CBS domain-containing proteins.

**Cystathionine \(\beta\)-Synthase**

Human CBS performs a crucial step in the biosynthetic pathway of cysteine by providing a regulatory control point for \(S\)-adenosylmethionine (AdoMet) (19). CBS catalyzes the condensation of L-serine and L-homocysteine, thereby forming L,L-cystathionine, which is subsequently cleaved to cysteine (for review, see Ref. 52). Alternatively, L-homocysteine, after being methylated to methionine, can be converted to AdoMet, which donates methyl groups to a variety of substrates, e.g., neurotransmitters, proteins, and nucleic acids. AdoMet functions as an allosteric activator of CBS by increasing the enzyme activity about threefold (20). In doing so, AdoMet exerts control on its biosynthesis: low concentrations of AdoMet result in low CBS activity, thereby funneling homocysteine in the transmethylation pathway toward AdoMet formation. In contrast, high AdoMet concentrations allow the clearance of homocysteine into the transsulfuration pathway, leading to cysteine biosynthesis (see Fig. 3A). It has recently been proposed (7) that CBS can also generate \(H_2S\), a putative paracrine modulator in the brain and the vascular system, via a condensation reaction involving cysteine and homocysteine.

Human CBS contains 551 amino acids and forms a homotetrameric enzyme complex in vivo (44) capable of associating into higher oligomers in solution (43). The COOH-terminal region of CBS encompasses a CBS domain (CBS1: amino acids 416–468), as originally described by Bateman (3), whereas sequence alignment with \(S. pyogenes\) IMPDH identified a second, less-conserved CBS domain (CBS2: amino acids 486–543) further down to the COOH terminus (69).

Several observations indicate that the COOH-terminal CBS domains are directly responsible for AdoMet binding and activation of enzyme activity. First, removal of the COOH-terminal CBS domains either by limited trypsinolysis or by site-directed mutagenesis generates a truncated CBS that is no longer activated by AdoMet (39). Consistent with this observation is the finding that \(Trypanosoma cruzi\) cystathionine \(\beta\)-synthase, which lacks the COOH-terminal CBS domains, is not activated by AdoMet (61). However, it should be pointed out that yeast cystathionine \(\beta\)-synthase, which has two CBS domains in its COOH terminus, is not regulated by AdoMet (47). Second, specific point mutations in the CBS domains of cystathionine \(\beta\)-synthase, e.g., D444N and S466L mutations in the first CBS domain, result in an AdoMet-insensitive enzyme (35, 40, 69). Third, the studies of Janosik et al. (35) revealed that the CBS domains form an autoinhibitory domain in cystathionine \(\beta\)-synthase, the effect of which is relieved by AdoMet binding. Fourth, recent work (68) involving binding...
assays with the isolated CBS pair of cystathionine β-synthase showed that AdoMet binds to the cystathionine β-synthase CBS pair with a dissociation constant of 34 μM. A very likely interpretation of these data is that the COOH-terminal CBS domains exert an autoinhibitory effect on CBS activity and that AdoMet binding to the CBS domains induces a conformational change, thereby relieving the autoinhibitory clamp.

In addition to their regulatory role, the CBS pairs in cystathionine β-synthase also affect the multimeric nature of the enzyme. Removal of the COOH-terminal CBS domains converts the human and yeast CBS homotetramer into a homodimer (37, 39) with a higher basal activity, but resistant to AdoMet activation (in the case of the human enzyme). Given the currently available structural data, it is unlikely that a CBS domain in one subunit interacts with a CBS domain in another subunit, but an association between two CBS pairs located in different subunits cannot be excluded and could contribute, either directly or indirectly, to the formation of a higher-order protein complex. However, structural analysis of the CBS complex is required to unequivocally elucidate the role of CBS domains/pairs in the tetramerization process.

CBS deficiency due to loss-of-function mutations in the CBS gene results in homocystinuria, a genetic disorder that is characterized biochemically by elevated plasma levels of homocysteine and clinically by mental retardation, lens dislocation, skeletal abnormalities, and endothelial dysfunction (52). A total of 131 different homocystinuria-causing mutations have been identified (see http://www.uchsc.edu/cbs/cbsdata/cbsmain.htm), some of which induce point mutation in either of the CBS domains: e.g., I435T, D444N, and S466L (40, 45, 69). A common functional feature of the mutations in the CBS domains is that they abolish or strongly reduce activation by AdoMet (40). Interestingly, these mutations can be discriminated based on their effect on AdoMet binding. The D444N mutation prevents AdoMet binding to the CBS domains (68), whereas the I435T and S466L mutants are still capable of AdoMet binding (35). Intriguingly, when the cystathionine β-synthase domains are modeled on the bacterial crystal structure, these mutations map to different locations, with respect to the central cleft. The D444 residue maps to the beginning of the first β-sheet and lines the central cleft. In contrast, I435 and S466 reside in the α1- and α2-helix, respectively, and do not contact the central cleft. Provided that the central cleft corresponds to the AdoMet binding site, the different positioning of the I435, D444, and S466 residues would nicely explain their opposing effects on AdoMet binding. At present it is not known how CBS domain mutations that do not interfere with AdoMet binding abolish enzyme activation. One possibility is that these mutations disturb the AdoMet-induced conformational change that leads to disinhibition of the enzyme.

AMP-Activated Protein Kinase

The AMPK cascade functions as a sensor of cellular energy content, and it plays an important role in restoring the cellular ATP balance during periods of metabolic stress (see Fig. 3B) (8). AMPK activation occurs when the cellular energy content drops (low ATP, high AMP) and requires a dual signal (see Fig. 4): binding of AMP and phosphorylation by an upstream kinase, the AMPK kinase (29). Once activated, AMPK switches on catabolic pathways (e.g., fatty acid oxidation, glycolysis) and switches off anabolic pathways (e.g., fatty acid and cholesterol synthesis, glycogen synthesis) by phosphorylation of regulatory metabolic enzymes in an effort to raise the cellular ATP level (for review, see Ref. 27). This wide range of effects not only affects the cellular energy management but also plays an important role in regulating whole body energy storage and expenditure. Recently, AMPK activity has been linked to type 2 diabetes and the metabolic syndrome, which has led to the concept of AMPK activators as new drugs to treat type 2 diabetes, obesity, and the metabolic syndrome (55, 77).

AMPK is a heterotrimer, composed of a catalytic subunit (α) and two regulatory subunits (β and γ). In mammals, different isoforms of each subunit have been identified: α1, α2, β1, β2, γ1, γ2, and γ1 (8, 73, 74). The γ-subunit contains four CBS domains, as shown in Fig. 4. Recently it has been reported that the two CBS pairs in the γ-subunit provide allosteric binding sites for AMP and ATP (68), as originally proposed by Daniel and Carling (15). The observation that both nucleotides, ATP as well as AMP, bind to AMPK is consistent with earlier

![Fig. 4. Model for the regulation of the AMP-activated protein kinase complex.](http://apcell.physiology.org)
The native enzyme is a homotetramer, and X-ray structure of the hamster type II IMPDH [which differs from human type II in only six amino acids (11, 59)] revealed two domains in the IMPDH monomer: a core domain, which forms an eight-stranded α/β barrel, and a subdomain, which consists of a CBS pair (71, 78). An identical structure in human IMPDH II was confirmed by Colby et al. (10). The core domains of the four subunits associate to form a central catalytic site, whereas the four CBS pairs are located at the periphery of the enzyme complex. Several observations indicate that the CBS pairs in the subdomain are dispensable for IMPDH function or tetramerization in vitro. First, a deletion mutant of human IMPDH II that lacks the CBS subdomain remains fully active in vitro (71). Second, Nimmesgern and coworkers (60) also showed that the tetrameric conformation is preserved in human IMPDH II that contains only the core domains. Nevertheless, mutations in the CBS domains of IMPDH I underlie an autosomal dominant form of retinitis pigmentosa (RP10), which suggests a critical in vivo function of these domains (5, 38).

Scott et al. (68) recently showed that the CBS pair of IMPDH II binds ATP in vitro and that the tetrameric IMPDH binds ATP in a positive, cooperative way (see Fig. 5). This observation can be explained by assuming that binding of the first ATP molecule to a CBS pair causes a conformational change, which increases the affinity for ATP of the remaining CBS pairs. They also observed that IMPDH was activated by ATP, which has never been reported before. ATP binding and activation was abolished by a single amino acid exchange (R224P) in the second CBS domain of IMPDH II. Interestingly, the R224P mutation corresponds to an RP10-causing mutation in IMPDH I (5). This provides strong evidence that ATP binding to the CBS domains allosterically activates IMPDH and consequently XMP synthesis (68). If so, this mechanism would couple the GTP/dGTP biosynthesis to the cellular energy status (high ATP levels).

**CLC Chloride Channels**

Chloride channels belonging to the CLC family sustain a wide variety of cellular functions, including membrane excitability, synaptic communication, transepithelial transport, cell volume regulation, cell proliferation, and acidification of endosomes and lysosomes (for review, see Ref. 36). The mammalian CLC family contains nine members (CLC-1–CLC-7, CLC-Ka, and CLC-Kb), which can be subdivided into three branches on the basis of sequence homology.

X-ray diffraction studies of bacterial CLC proteins have revealed a dimeric two-pore structure in which each pore is formed by a single subunit (17). In view of the sequence identity between prokaryote and eukaryote CLC proteins and in view of functional evidence consistent with two independent conduction pathways in mammalian CLC-1 (65), it is very likely that eukaryotic CLC proteins adopt a dimeric, double-barreled structure. In contrast to bacterial CLC proteins, which have a short COOH terminus without CBS domains (17, 48, 54), all eukaryotic CLC proteins possess a long COOH-terminai cytoplasmic region that contains two CBS domains (3, 62). Because bacterial CLC proteins dimerize in the absence of CBS domains (48, 54), it can be inferred that the CBS domains are not required for dimerization. Experiments with COOH-terminal truncation mutants of mammalian CLC-1 have indeed
confirmed that the CBS domains are dispensable for protein dimerization (18). Furthermore, because the CBS domains reside in the cytosol, they do not contribute to the core structure of the permeation pathway, which is formed by a subset of the membrane-embedded α-helices A to R. Although the CBS domains are located outside the membrane-spanning part of the channel, they may still modulate key properties such as channel gating (see below). Indeed, the cytosolic COOH terminus containing the CBS domains is an extension of the ultimate membrane helix R, which is part of the selectivity filter (17). This structural link allows for the direct transmission of interactions at the CBS domains to the channel pore. Finally, it should be mentioned that the CBS1 and CBS2 domains are separated by an intervening region, which varies in length between the different CLC isoforms. Whether this intervening region affects the function of the CBS domains remains to be shown.

The functional role of CBS domains in CLC channel function remains largely unresolved and even controversial. However, it is clear that CBS domains in human CLC channels are required for CLC function and/or expression because mutations in CBS domains of CIC-1, -2, -5, and -7 and CIC-Kb result in specific diseases caused by CLC dysfunction (9, 28, 41, 42, 46, 64). The requirement for intact CBS domains is also corroborated by expression studies of CIC-0 or CIC-1 channels lacking the second CBS domain. Expression of CBS2-deficient truncation mutants in *Xenopus laevis* oocytes failed to generate typical CIC-0 or CIC-1 membrane currents, whereas coexpression of the truncated proteins with the CBS2-containing COOH-terminal fragment restored channel function (49, 66).

One set of data implicates CBS domains in the intracellular trafficking of CLC proteins. First, when expressing mouse CIC-5 mutants in CHO-K1 and IMCD-3 cells, Carr et al. (6) discovered that truncation mutants with a disrupted second CBS domain are retained in a perinuclear compartment instead of being delivered to acidic endosomes as was the fate of wild-type CIC-5. Second, disruption of the second CBS domain by alanine scanning led to mislocalization of the *Saccharomyces cerevisiae* CLC (Gef1p) (67). Wild-type Gef1p is sorted to the Golgi apparatus, but Gef1p with a mutated CBS2 is retained in a compartment that also stains for Kar2p, a marker for the endoplasmic reticulum. Third, it was recently shown in expression studies in *Xenopus laevis* oocytes that CIC-1 proteins truncated after the first CBS domain did not result in specific diseases caused by CLC dysfunction (9, 28, 41, 42, 46, 64). The requirement for intact CBS domains is also corroborated by expression studies of CIC-0 or CIC-1 channels lacking the second CBS domain. Expression of CBS2-deficient truncation mutants in *Xenopus laevis* oocytes failed to generate typical CIC-0 or CIC-1 membrane currents, whereas coexpression of the truncated proteins with the CBS2-containing COOH-terminal fragment restored channel function (49, 66).

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### Table: Overview of the CBS domains

<table>
<thead>
<tr>
<th>CBS domains</th>
<th>Proposed function CBS domains</th>
<th>CBS-associated pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystathionine β-synthase</td>
<td>• AdoMet binding • Multimerization</td>
<td>• Homocystinuria OMIM 236200</td>
</tr>
<tr>
<td>AMPK γ2-subunit</td>
<td>• AMP/ATP binding</td>
<td>• Familial hypertrophic cardiomyopathy with WPW syndrome OMIM 600568</td>
</tr>
<tr>
<td>IMPDH</td>
<td>• ATP binding • Regulatory protein binding</td>
<td>• Retinitis Pigmentosa OMIM 160105</td>
</tr>
<tr>
<td>CLC channels</td>
<td>• Trafficking? • Gating? • ATP binding? • Regulatory protein binding?</td>
<td>• Myotonia (CIC-1) OMIM 160800 • Idiopathic generalized epilepsy (CIC-2) OMIM 600069 • Dan’s disease (CIC-5) OMIM 300009 • Osteopetrosis (CIC-7) OMIM 259700 • Bartter Syndrome (CIC-Kb) OMIM 241200</td>
</tr>
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**Fig. 5.** Overview of the CBS domains. WPW, Wolff-Parkinson-White; CLC, chloride channel; and OMIM, Online Mendelian Inheritance in Man.
reach the plasma membrane and that coexpression with the COOH-terminal fragment containing the second CBS domains restored plasma membrane delivery (18). Functional reconstitution and hence plasma membrane delivery of the split CIC-1 channel was observed only when the COOH-terminal fragment contained an intact CBS2 domain (18). However, one should be cautious in deducing from these data that CBS2 contains a sorting signal for plasma membrane delivery. Indeed, in-frame deletion of CBS2 did not affect the functional expression of CIC-1 channels, indicating that CBS2 as such is dispensable for membrane sorting and channel function (18, 31). One interpretation of these data is that CIC-1 contains an as yet uncharacterized sorting signal for plasma membrane delivery or retention that does not reside in the CBS2 domain and that functions both in the single peptide channel and in the split channel configuration. However, in the split channel, the two fragments must associate via a CBS1-CBS2 interaction to bring the sorting signal into a “sorting-competent” position. In contrast, in the single peptide channel, the sorting signal is correctly positioned independently of the CBS interaction. That the CBS domains in CIC-1 do not contain a plasma membrane sorting signal sensu stricto can also be concluded from the experiments reported by Hebeisen et al. (30). In contrast to Estevez et al. (18), they observed that COOH-terminal truncation mutants of CIC-1, when overexpressed in tsA201 cells (a SV40-transformed variant of human embryonic kidney HEK-293), were inserted into the plasma membrane even if they lacked CBS domains. To conclude, although some of the available data suggest that CBS domains influence, either directly or indirectly, the subcellular localization of CLC proteins, it is still too early to draw any firm conclusion about the underlying mechanism. Finally, one should always bear in mind that the missorting of a mutant protein does not necessarily reflect the loss of a sorting signal but could be secondary to malfolding of the mutant protein and retention of the malfolded protein by the quality control system in the endoplasmic reticulum.

A second set of data suggests that CBS domains may also affect the functional properties of CLC channels. For example, Estevez et al. (18) identified specific point mutations in the CBS2 domain that changed the common slow gate of CIC-1 and CIC-0. In particular, it was shown that mutation of E763, which corresponds to a conserved acidic residue in many CBS domains, abolished the common slow gate of CIC-0 without interfering with the fast gate of the individual protopores. These experiments are consistent with previous observations, which revealed that exchanging the CBS domains between, on the one hand, CIC-0, and on the other hand, CIC-1 or CIC-2, modified the common gate of CIC-0 (21). Consistent with a role for CBS domains in channel function, Hebeisen et al. (30) reported that CBS domains are required for proper functioning of the CIC-1 channel. However, in contrast to Estevez et al., they concluded that CBS domains do not (or only minimally) affect the gating behavior of CIC-1 and that the CBS domains act only in cis, i.e., on the corresponding protopore and not on the other protopore of the channel dimer. Finally, Hebeisen et al. also concluded that CBS1 and CBS2 do not bind to each other and that a single CBS domain is sufficient to support CIC-1 channel function.

At first sight, the studies by Estevez et al. and Hebeisen et al. offer some divergent views on the role of CBS domains in sorting and gating of CIC-1, but it is presently not clear how these discrepancies should be resolved. For the sake of clarity, it should be mentioned that both data sets were obtained under different experimental conditions. Hebeisen et al. transiently overexpressed wild-type and mutant CIC-1 (either “naked” or as fusion protein with yellow/cyan fluorescent protein) in tsA201 cells. In contrast, Estevez et al. overexpressed wild-type and mutant CIC-1/0 with or without epitope tags in Xenopus oocytes. However, it remains to be established to which extent the different methodologies can explain the apparent differences between both studies. Nevertheless, a couple of common conclusions can be deduced despite the partially incongruent data. First, formation of a COOH-terminal CBS pair is not required for plasma membrane delivery of CIC-1. Indeed, either CBS1 (30) or CBS2 (18) can be in-frame deleted without compromising surface expression of the mutant channel, indicating that the critical sorting signal for plasma membrane delivery is not located within the CBS pair. Second, the CBS domains in CIC-1 function as interchangeable units because CBS1 or CBS2 could be replaced by CBS domains of other CLC channels or of other CBS-containing proteins. This is consistent with the modular structure of a CBS pair as revealed by X-ray diffraction of bacterial CBS proteins. Third, CBS domains in CIC-1 modulate the functional behavior of the channel.

Finally, an unresolved question with respect to the CBS domains in CLC channels is whether they bind any ligands (small molecules, proteins) and, if so, whether this has any functional implications. On the basis of an in vitro binding assay using bacterially expressed CBS pairs, Scott et al. (68) proposed that ATP is the natural ligand for CLC CBS pairs. Because the CBS domains of all CLC channels are predicted to reside in the cytosol, Scott et al. (68) hypothesized that CLC CBS pairs function as cellular energy/ATP sensors and that ATP binding to the CLC CBS domains is required for channel gating. Interestingly, mutations in the CIC-2 CBS domains, e.g., G715E (associated with generalized epilepsy, see Ref. 28) and G826D (equivalent to a CIC-1 mutation causing congenital myotonia, see Ref. 64), strongly diminish the in vitro ATP binding, suggesting a causal link between reduced ATP binding and channel dysfunction. However, it remains unclear whether ATP binding is a universal property of all CLC CBS domains and, if so, to which extent ATP binding modulates the functional properties of CLC channels. Of note, there is some circumstantial evidence in favor of this hypothesis. For example, human CIC-4 only generates a CLC when ATP or a nonhydrolyzable analog is provided at the cytoplasmatic side (75), but it remains to be shown that ATP mediates its effect via binding to one of the CIC-4 CBS domains.

In conclusion, intramolecular association of two CBS domains generates a CBS pair with a conserved tertiary structure in spite of the rather low sequence identity of individual CBS domains. The bacterial crystal structures reveal a cleft between the two CBS domains, which may correspond to the binding site for the various ligands (ATP, AMP, AdoMet) that have been reported in in vitro studies of mammalian CBS domains. In vivo corroboration of the ligand binding properties is required, but the hypothesis that CBS domains function as metabolic sensors by sampling the cytosolic AdoMet, ATP, and/or AMP levels provides an interesting and unifying concept that couples domain function to the specific function of the
protein in which the CBS domain resides. Such a metabolic sensor function would also explain the occurrence of equivalent disease mutations in human CBS proteins. For example, the D444N mutation in cystathionine-β-synthase, the R302Q mutation in the γ-subunit of AMPK and the R767Q or R767W mutations in CLC-7 mutations in CLC-7 affect a structurally equivalent residue corresponding to serine at position 122 in S. pyogenes IMPDH. Because S122 in the bacterial IMPDH lines the upper part of the cleft in the CBS pair, it is an interesting hypothesis that the above-mentioned mutations cause dysfunctional CBS domains by interfering with binding of the endogenous ligand (68).

However, the metabolic sensor concept raises several questions. First, it is still an open question whether sampling of cytosolic metabolites is the only function of CBS domains. In particular, it remains to be elucidated what the precise role of CBS domains in CLC channels is and whether the observed effects on channel function and/or sorting require the binding of a cytosolic ligand to the CLC CBS domain. A second question relates to the molecular mechanisms, which is responsible for the communication between the CBS domain and the functional core of the protein. Additional experiments are required to understand how ligand binding or dissociation alters the enzymatic activity or the channel behavior. A third question (and also a challenge) is whether molecules can be developed to be targeted specifically to the CBS domains of AMPK (as antidiabetic or anti-obesity drugs) or of IMPDH (immunosuppressive or anticancer therapy).

NOTE ADDED IN PROOF After this review was accepted, a paper was published which shows that ATP inhibits CIC-1 by shifting the voltage dependence and that specific mutations in the CBS domains abolish or reduce the ATP inhibition (3a).

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