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The CBS Domain: A Protein Module with an Emerging Prominent Role in Regulation

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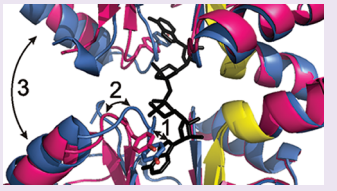
ABSTRACT: Regulatory CBS (cystathionine β-synthase) domains exist as two or four tandem copies in thousands of cytosolic and membrane-associated proteins from all kingdoms of life. Mutations in the CBS domains of human enzymes and membrane channels are associated with an array of hereditary diseases. Four CBS domains encoded within a single polypeptide or two identical polypeptides (each having a pair of CBS domains at the subunit interface) form a highly conserved disk-like structure. CBS domains act as autoinhibitory regulatory units in some proteins and activate or further inhibit protein function upon binding to adenosine nucleotides (AMP, ADP, ATP, *S*-adenosyl methionine, NAD, diadenosine polyphosphates). As a result of the differential effects of the nucleotides, CBS domain-containing proteins can sense cell energy levels. Significant conformational changes are induced in CBS domains by bound ligands, highlighting the structural basis for their effects.



CBS domains (Pfam: PF00571[1]) are small protein modules (typically 60 residues in length) originally identified by Alexander Bateman in human cystathionine β-synthase and several bacterial proteins[2] and subsequently found in thousands of cytosolic and membrane-associated proteins from all kingdoms of life.[1] For instance, 75 CBS domain-containing proteins exist in *Homo sapiens* and 8 such proteins in *Escherichia coli* according to the Pfam database. CBS domains are added as structurally separate units to functional domains in characterized proteins. Proteins consisting of CBS domains only have additionally been identified, particularly in prokaryotes. However, the functions of these "minimal" proteins are yet to be determined.

Point or deletion mutations in CBS domains of several human enzymes and membrane channels are associated with hereditary diseases, including homocystinuria, retinitis pigmentosa, Bartter syndrome, osteopetrosis, Dent disease, Wolff-Parkinson-White syndrome, and familial hypertrophic cardiomyopathy.[3] These proteins, including AMP-activated protein kinase (AMPK), cystathionine β-synthase (CBS), inosine-5′-monophosphate dehydrogenase (IMPDH), and chloride-conducting channel (ClC), are the most extensively characterized among the CBS domain-containing proteins with known functions. Recent investigations have further uncovered bacterial Mg^{2+} transporter (MgtE),[4] ABC glycine betaine transporter (OpuA),[5] and inorganic pyrophosphatase (CBS-PPase)[6] as part of this list.

AMPK is a heterotrimeric protein containing a tetra repeat of CBS domains within its γ subunit.[7] Mammals contain three paralogous γ subunits solely comprising CBS domains, among which γ1 is the shortest. Other enzymes and transporters specified above contain a pair of CBS domains. Cystathionine β-synthase is a homodimeric/tetrameric protein with the regulatory heme domain located at the N-terminus, catalytic part in the middle, and CBS pair at the C-terminus.[8] Two paralogous IMPDHs are homotetrameric proteins with the CBS pair inserted within the catalytic sequence.[9] Nine paralogous forms of mammalian ClC[10] and bacterial ClC[11] and MgtE[12] are homodimeric integral membrane proteins with a CBS domain pair in the cytoplasmic region. Bacterial OpuA is a heterotetramer containing a cytosolically oriented CBS domain pair in each of the two nucleotide-binding OpuAA subunits.[13] Homodimeric CBS-PPase contains two CBS domains within the catalytic sequence.[6]

Here, we have reviewed the progress made over the past decade in understanding the regulatory role of CBS domains in these proteins, including (a) the structure of CBS domains within the context of whole protein structures, (b) physiological ligands of the CBS domains, (c) ligand-induced conformational changes, and (d) communication between CBS and functional domains. The medical significance of CBS domain proteins has been covered by a survey of Ignoul and Eggermont.[3] A brief review on CBS domains by Kemp[14] during the early stages of research has additionally been published.

STRUCTURE OF CBS DOMAINS

CBS domains have low sequence conservation within protein families, and even within the same protein (Figure 1), but are uniform in length. These domains are usually adjacent to each other, but separated by a DRTGG domain (Pfam: PF07085[1]) in approximately 1% of protein sequences (mostly bacterial CBS-PPases). CBS domains usually occur in pairs and less frequently in quads. However, proteins containing 1, 3, 5, 6, 7, or ultimately 14 copies have also been identified.[1] CBS domain pairs form a tight structure, alternatively called a Bateman domain or Bateman module. The latter designation is more appropriate in our

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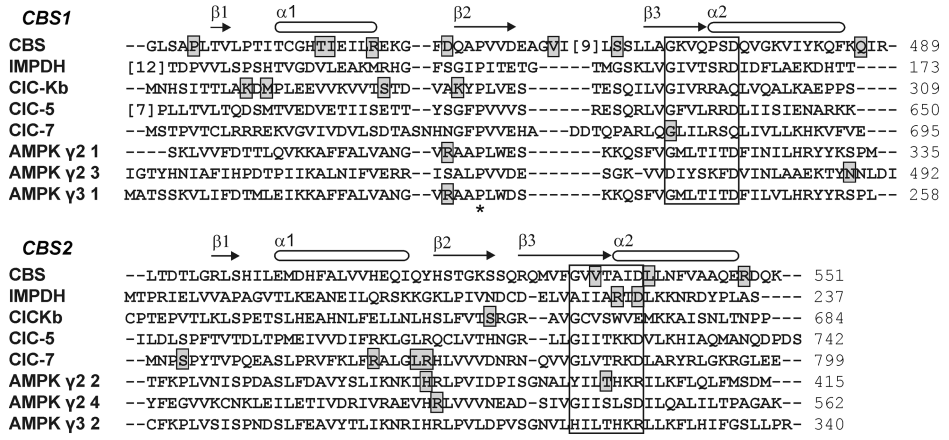

Figure 1. Sequence alignment of CBS domains 1 and 2 of human proteins with disease-causing mutations (shaded in gray). For AMPKs containing two pairs of CBS domains per subunit, CBS domain numbers are presented after protein names. Sequence extensions are indicated as the number of residues in parentheses. Sequence numbering is shown on the right. The most conserved CBS motifs are presented in boxes. A single Pro residue (marked with an asterisk below the sequences) is conserved throughout the whole protein set.

opinion. Interestingly, these domains occur more frequently as a single structure than a pair in plant proteins.[15]

Crystallization of CBS domain-containing proteins is a significant challenge, and full-length 3D structures have been determined for few multidomain proteins. However, crystallization attempts with separate CBS domain fragments and proteins composed of CBS domains only have been more successful to date. It appears that CBS domain-containing proteins are flexible, especially in their functional domains, as expected for regulatory proteins, whereby a small change induced by the effector in the regulatory part triggers a more significant change in the functional region.

The first full-length structure, specifically, IMPDH from *Streptococcus pyogenes*, was determined in 1999.[9] More recently, these structures were reported for the bacterial Mg^{2+} transporter MgtE,[12] *Drosophila* cystathionine β-synthase,[16] and red alga *Cyanidioschyzon merolae* ClC transporter.[11] Additionally, the structure of the zinc ribbon-like protein, TA0289, with unknown function has been solved.[17] The structures show that Bateman modules form separate units on the protein surface and display conserved three-dimensional structures, despite low sequence similarity. CBS domains fold into an α/β structure with internal symmetry. They consist of a typical β-α-β-β-α core, an additional small helix, and often a short β-strand at the N-terminal region (Figure 2) that forms a link to the neighboring CBS domain. Potential ligand-binding sites are located in interdomain cavities. The Bateman module is a tight structure, since its constituent domains complement each other, even when expressed separately[19] or derived from different proteins.[20]

Although crystallization of complete AMPK has not been successful to date, core structures, including the γ1 subunit and closely related parts of α and β subunits, have been solved.[21−23] The most recent structure of an active hybrid rat/human AMPK complex displays complete γ1, the majority of α, and a smaller part of β subunits.[24] Tetra repeats of the CBS domain found in the γ1 subunit are organized into a disk-like structure (Figure 2c) about 60 Å in diameter and 30 Å in width. The CBS domain pairs are assembled in a head-to-head manner, making one contact through β-sheets and the other through α-helices, whereby CBS1 interacts with CBS3 and CBS2 with CBS4.

Another group of structures includes the fragments of larger proteins containing CBS domain pairs or quads, such as the Mg^{2+} transporter MgtE,[12] the chloride channels ClC-0,[25] ClC-5,[26] and ClC-Ka,[27] CBS-PPase,[6] a separate γ1 subunit of AMPK,[21,22,28,29] CcpN repressor (PDB IDs: 3FWS, SFWR, 3FV6), as well as the MJ0100 protein with unknown function from *Methanocaldococcus jannaschii*.[30] These structures are similar to those in the corresponding full-length proteins, where available, signifying the self-sufficiency of CBS domains in protein structures.

A third group includes proteins comprising CBS domains only with unknown functions. Many of these proteins were predicted on the basis of open reading frames from various bacterial genome projects and are known only by their gene numbers. Some of the corresponding structures are available at PDB but are accompanied by journal papers in some cases.[30−37] A conventional Bateman module has been identified in these proteins, with several exceptions. Two protein structures contain quads of CBS domains (MJ1225 from *M. jannaschii* [31] and Sso3205 from *Sulfolobus solfataricus* (3DDJ)) organized in a way similar to the AMPK γ1 subunit. In the ST2348 protein from *Sulfolobus tokodaii*, four CBS pairs form a cyclic structure *via* helix/helix contacts.[35]

The majority of CBS domain-containing proteins form homodimers, with the CBS domains participating in subunit interface interactions. Consequently, the two CBS domain pairs typically form a disk-like structure (baptized as "CBS module" by Mahmood *et al.*[38]) similar to that found in the AMPK γ1 subunit. However, there are several notable exceptions to this oligomerization pattern. For instance, while the majority of proteins display head-to-head assembly with the dimer interface involving both CBS domains, TM0935 from *Thermotoga maritima* exhibits a head-to-tail assembly.[37] Furthermore, dimerization specifically involves the CBS2 domains in the chloride channels ClC[25,27,39] and the ST2348[35] protein or CBS1 domains in the hypoxic response protein 1 of *Mycobacterium tuberculosis*.[36] Such assembly adopts a V-shape structure[11] distinct from the disk-like appearance. Notably, neither CBS domain participates in oligomerization in IMPDH from *S. pyogenes*.[9]

CBS DOMAINS AS REGULATORY UNITS

The Bateman module may be absent in orthologous proteins.[40−42] Furthermore, its deletion in cystathionine β-synthase[8] and

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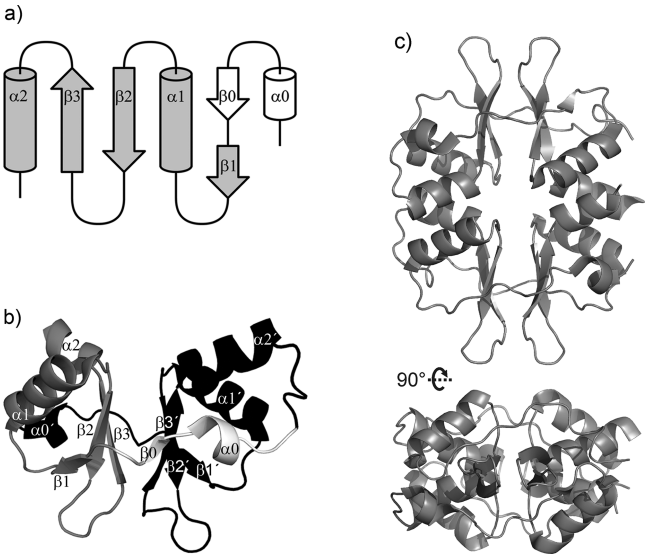

Figure 2. (a) Topology of the CBS domain. The CBS domain core is presented in gray, and the common CBS linker is in white. (b) 3D structure of the CBS domain pair in PAE2072 from *Pyrobaculum aerophilum* (PDB ID: 2RIF). Different CBS domains are colored in gray and black. $\beta 0$ forms a common β-sheet with $\beta 1'-\beta 3'$ of the pairing CBS domain. (c) Two projections of the 3D structure of the CBS domain tetra repeat in the AMPK $\gamma 1$ subunit (PDB ID: 2OOX). The figure was created with Pymol and TopDraw.[18]

IMPDH[43] does not affect (or in some cases increases) their catalytic activities. These findings indicate that CBS domains are not directly involved in catalytic function. CBS domains only appear essential for function (and cellular localization) in ClCs,[44,45] which are membrane proteins with more complex relationships between the cytosolical and membrane-bound parts.

Most CBS domains bind adenine nucleotides as regulatory ligands. The first CBS domain ligand was identified by Kery *et al.*,[46] who showed that CBS activation by *S*-adenosyl methionine (AdoMet) was prevented upon deletion of C-terminal CBS domains. In a breakthrough study, Scott *et al.*[47] performed direct binding measurements that demonstrated AMP and ATP interactions with isolated CBS domains of AMPK, IMPDH, and ClC-2, AdoMet binding to CBS, and their impairment following disease-related mutations. Binding constants generally vary in the range of 0.01−10 mM,[47] although values as low as 0.01 μM are not uncommon.[42]

Ligand binding studies have facilitated the determination of adenylate-bound structures. The CBS domain nucleotide ligands identified *via* X-ray crystallography to date include AMP [6,22,23,28,31,34,48] (PDB IDs: 3NQR, 3LHH, 3LFR, 3HF7, 3FNA, 3FHM, 3DDJ), ADP [21,23,31,39] (PDB ID: 3FWR), ATP,[22,23,39] diadenosine polyphosphate,[6] NADH,[24,33] AdoMet [30] (PDB ID: 2YZQ), and 5′-deoxy-5′-methylthioadenosine.[30] Furthermore, some structures contain bound adenine nucleotide analogues (adenosine 5′-(β,γ-imido)-triphosphate (PDB ID: 3FWS) and 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide-5′-monophosphate (ZMP)).[23,28]

Nucleotides bind to the cleft within the Bateman module containing some conserved elements, for instance, a ribose phosphate-binding motif Ghx(T/S)x(T/S)D (where h is a hydrophobic residue and x any residue).[28] The hydrophobic residue (h) interacts with the adenine ring, both Thr/Ser residues bind

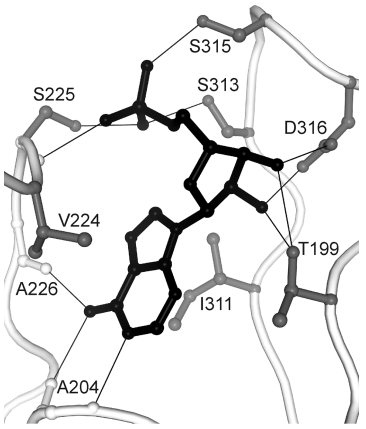

Figure 3

Figure 3. Binding contacts of tightly bound AMP in AMPK $\gamma 1$ subunit (PDB ID: 2V8Q). Protein backbone is presented in white, side chains are in gray, and AMP is in black.

phosphate, and aspartate binds ribose hydroxyls (Figure 3). The backbone atoms of the hydrophobic residue contact adenine N1 (-N) and N6 (-O). The pairing CBS domain provides the other hydrophobic residue to interact with the adenine ring. The notable variations in the binding residues may explain the diversity of CBS domain ligands. The protein MJ1225 from *M. jannaschii* additionally contains a noncanonical AMP binding site located in a small cavity between the α-helices.[31]

Each CBS domain contains a potential binding cavity for nucleotides, yet a Bateman module typically binds only one adenylate ligand. However, mammalian AMPK $\gamma 1$ binds nucleotides at three potential sites,[22,24] whereas AMPK $\gamma 1$ from *Schizosaccharomyces pombe* [23] and MJ1225 from *M. jannaschii* [31] bind four nucleotide ligands to all possible sites in the CBS domain tetra repeat. The binding affinities for the sites are variable. *Rattus norvegicus* AMPK $\gamma 1$ contains one strong AMP binding site at the CBS4 domain, which maintains interactions with the ligand through the protein purification process or soaking with other adenylate nucleotides.[22] The same site binds the drug molecule, ZMP.[23,28] The exchangeable sites of AMPK $\gamma 1$ also differ in terms of binding affinity.[24] MgATP, the predominant form of ATP in cells, interacts with these sites in AMPK with 10-fold lower affinity, in comparison with free ATP.[24] Two Bateman modules of dimeric *M. thermoacetica* CBS-PPase show strong negative cooperativity in AMP analogue binding.[49]

However, adenine nucleotides are not the universal ligands for CBS domains in all proteins, which may instead bind metal ions or nucleic acids. Several Mg^{2+} ions are bound to the interface between cytosolic CBS and transmembrane domains in the structure of *T. thermophilus* MgtE.[4,12] Bound Mg^{2+} ions have also been found in a *Methanothermobacter thermautotrophicus* protein (PDB ID: 1PBJ) and bound Zn^{2+} ions in *M. tuberculosis* HRP1.[36] Moreover, single-stranded DNA and RNA binding to the CBS domains of bacterial IMPDH has been demonstrated *in vitro* and *in vivo*[50] and a role for this interaction in translation regulation suggested.[51] Aguado-Llera *et al.* recently reported double-stranded DNA binding to the CBS domain-only protein, MJ0729, from *M. jannaschii*.[52]

The CBS domain insert sensitizes proteins with catalytic or transport functions to structural changes caused by nucleotide binding by acting as an "internal inhibitor", permitting either

activation or further inhibition. This theory is consistent with the following observations. First, cystathionine β-synthase[8] is activated upon deletion of the C-terminal CBS domain-containing region[46] or AdoMet binding. Second, CBS domain-containing pyrophosphatases are less active by 2 or 3 orders of magnitude, compared with their close homologues lacking CBS domains.[42] Third, many point mutations in the CBS domains of both enzymes, [46,53] as well as rat AMPK γ1[54] and pig AMPK γ3,[55] lead to activation of these enzymes. Finally, AMPK[24] and CBS-PPase[42] can be activated or inhibited, depending on the bound nucleotide identity. Moreover, the effects of the nucleotides on CBS-PPase can be reversed by appropriate mutations in the CBS domain.[53]

AMPK is regulated in a unique manner, whereby AMP and ATP exert dual effects. This enzyme is activated several hundred-fold upon phosphorylation of α-subunit Thr172 by upstream protein kinases.[56] In addition to its allosteric effect on the active site (resulting in 2- to 5-fold activation),[57] AMP binding to the regulatory γ subunit promotes phosphorylation and inhibits dephosphorylation of Thr172, thus maintaining AMPK in its most active form.[58] ADP behaves similarly in the phosphorylation/dephosphorylation reactions but does not directly activate AMPK.[59] ATP inhibits AMPK allosterically and promotes dephosphorylation of Thr172.[24,59] Interestingly, the effects of AMP and ADP on Thr172 phosphorylation are observed only when the β subunit of AMPK is N-terminally myristoylated.[58,59] ClC is inhibited by phosphorylation of two adjacent Ser residues,[61] but the role of CBS domains in this protein is not well-defined. In contrast, activation of cystathionine β-synthase by AdoMet may simply result from stabilization of intersubunit contacts, in which CBS domains as well as the linker connecting the CBS and catalytic domains are involved.[16]

The direction of the effect (inhibition or activation) of a particular nucleotide depends on the protein identity. Whereas ClC-4 [62] and ClC-5 [63] are activated by ATP, ClC-1[64] and the plant ClC family member *Arabidopsis thaliana* nitrate/proton antiporter (AtClCa) are inhibited by this nucleotide.[65] AMP and ADP similarly activate ClC-5[63] and inhibit ClC-1[64] but have no effect on AtClCa activity.[65] As mentioned above, mammalian AMPK is allosterically activated by AMP and inhibited by ATP, whereas ADP has no effect.[24] CBS-PPase is inhibited by AMP and ADP but activated by ATP and diadenosine polyphosphate.[6,42]

Differential actions of adenine nucleotides allow the respective CBS domain-containing proteins to sense cell energy levels. When the ATP level decreases, ADP and, particularly, AMP levels increase. Since adenylate kinase maintains a constant ATP:ADP ratio, the AMP concentration displays the largest variations during cell stress, and therefore AMP is a more effective sensor molecule than ADP.[66] AMPK has been extensively characterized in this respect and functions as a whole human body energy balance sensor by phosphorylating numerous downstream substrates.[67] CBS-PPase inhibition by ADP and AMP provides an interesting mechanism for switching from an ATP-dependent to ancient PP_i-dependent metabolism[68] at low ATP levels in bacteria containing PP_i-energized H^+ and Na^+ pumps.[69] Genome sequence analyses have disclosed that such pumps are common partners of CBS-PPases but never occur together with unregulated family II PPases.

Several CBS domain-containing proteins appear to sense ionic strength rather than nucleotide concentration. The *Lactococcus lactis* ABC transporter, OpuA, protects cells against hyperosmotic stress through ATP-supported uptake of the compatible solute glycine betaine accompanied by water influx. OpuA is deactivated at low internal ionic strengths and activated above a threshold ionic strength. Deletion of the CBS2 domain makes the protein almost insensitive to ionic strength.[5] Earlier, Mahmood *et al.*[38] showed that positively charged residues located in a loop between β2 and β3 of the CBS domains (Figure 2) are essential for ionic strength sensing. ATP is bound to and hydrolyzed by the NBD domain of OpuA, but no nucleotide appears to interact with the CBS domains. Increased ionic strength and alkaline stress conditions activate *S. cerevisiae* AMPK, and this effect does not appear to be mediated by adenine nucleotides.[70]

Another membrane protein, MgtE, senses Mg^{2+} concentrations within the cell. MgtE is a specific Mg^{2+} membrane channel containing a pair of CBS domains in the cytoplasmic region. As evident from the structure of *Thermus thermophilus* MgtE, binding of several Mg^{2+} ions to the CBS domains induces large conformational changes, acting as a gating mechanism for the functional transmembrane domain.[4,12]

STRUCTURAL BASIS FOR REGULATION

While the theory that CBS domains regulate the activity of associated functional domains has become a dogma, the molecular mechanisms underlying regulation have only just begun to emerge. A widely accepted way to clarify the regulation mechanisms is to compare protein structures in the presence and absence of the regulating ligand or with activating and inhibiting ligands. Because of difficulties in crystallizing both forms, these structural pairs have become available only recently. MgtE is the first protein for which large structural changes have been documented upon ligand binding to the CBS domains.[12] In the Mg^{2+}-free form, the two cytosolic CBS domains are separated from each other by a 40° rotation ("open" conformation), and the cytosolic N domain rotates away from the CBS domain by 120°, compared to the Mg^{2+}-bound form. These structural changes provide a gating mechanism for the ion-conducting pore in the membrane.[4] MgtE appears to be unique in its use of Mg^{2+} as regulator, since most other CBS domain-containing proteins utilize adenine nucleotides in this capacity.

Nucleotide binding induces large-scale conformational changes in the regulatory region of *C. perfringens* CBS-PPase [6] and CBS domains of the protein MJ0100 with unknown function from *M. jannaschii*.[30] CBS-PPase structures containing bound AMP (inhibitor) and AP_4A (activator) were compared (Figure 4). The AMP structure contained two nucleotide molecules per CBS-PPase dimer (one per Bateman module), whereas only one nucleotide molecule occupied both AMP binding sites in the AP_4A structure. The CBS2 domains of the two structures superimposed with rmsd of 2.3 Å, whereas the rmsd value was only 0.7 Å for the rest of the structure. A clear conformational transition was evident, where different orientations of the adenosine rings of the activator led to displacement of Tyr278 and the whole $RY^{278}SN$ loop and, consequently, a more open CBS2 domain dimer interface (Figure 4). The transition from the nucleotide-free to nucleotide-bound state involved three steps with relaxation times from 0.01 to 100 s, measured using the stopped-flow method with a fluorescent AMP analogue.[49] Looser packing of the AP_4A-bound structure was substantiated by its significantly lower thermostability, compared with the AMP-bound structure.[6]

Structures of the protein MJ0100 were determined with different combinations of bound AdoMet and *S*-methyl-5′-thioadenosine, which induced distinct conformations.[30] The deduced

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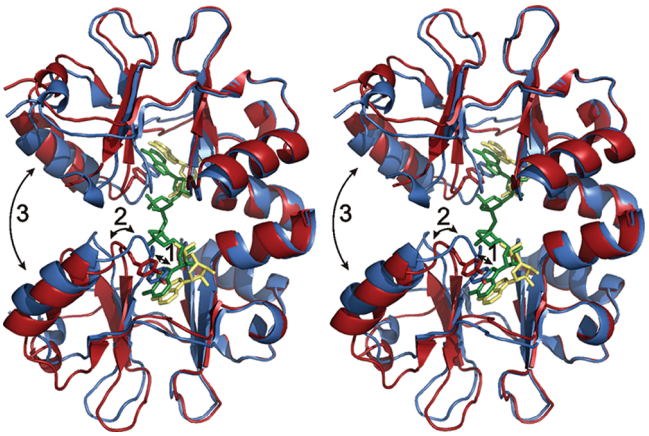

Figure 4. Stereoview of superimposed AMP- (blue) and AP_4A-bound (red) structures of the regulatory region of *C. perfringens* pyrophosphatase (PDB IDs: 3L31 and 3L2B). AMP and AP_4A molecules are shown in yellow and green, respectively. The numbers represent movement of Tyr278 along aromatic interactions to the adenine ring of ligand (1), conformational change of the RYSN loop (2), and opening the CBS2 dimer interface in the AP_4A complex (3). Taken with permission from Tuominen *et al.*[6]

binding scheme assumes that AdoMet binds initially in such a way that the dimer interface is 23° open. Subsequently, AdoMet adopts a more compact conformation, allowing the binding of a second AdoMet molecule (interface 12° open). In the third structure, both AdoMet molecules are ordered (interface 9° open), facilitating the final binding of one or two 5-methyl-5′-thioadenosine molecules and dimer closure. Analogous to CBS-PPase, the conformational changes mostly involved the CBS2 domain interface. Moreover, similar displacement of a Phe residue from the dimer interface to the CBS domain interior was observed in activated CBS-PPase and 12°- and 23°-opened MJ0100.

On the other hand, only minor differences were observed between the structures of the regulatory part of ClC-5 bound to ATP and ADP.[39] However, this is expected, as both nucleotides act as activators and should therefore display structural alterations in the same direction.

Indirect methods have also been employed to monitor structural changes in CBS domains. Martínez-Cruz *et al.* used FTIR, FRET, and fluorescence measurements to detect nucleotide-induced structural changes in the CBS domain-only protein MJ0729 of *M. jannaschii*.[71] Hnízda and co-workers measured the accessibility of CBS to proteolysis and chemical modifications, with a view to identifying the residues undergoing the most significant movement upon AdoMet binding.[72] FRET microscopy disclosed a significant movement in ClC-0,[73] although the regulator is yet to be identified.

The conformational flexibility of the CBS domains is thus well documented. However, the mechanisms by which structural changes are transmitted to functional domains are poorly understood. This problem was only partially resolved with unmyristoylated AMPK, which has been characterized extensively with regard to the effect of the CBS domain on the degree of α-subunit Thr172 phosphorylation.[24] The equilibrium of the phosphorylation reaction is determined by the accessibility of the phosphorylation site to respective protein kinases and protein phosphatases. AMP and ADP binding to one of the four potential binding sites in the CBS domain quad of the γ subunit prevents dephosphorylation, thereby shifting the equilibrium to the phosphorylated active enzyme, but not ATP binding.[24,60] The effect is attributed to the size of the bound nucleotide, which sterically controls the conformation of a short segment (residues 373−382) in the catalytic α-subunit. This segment, in turn, controls interactions of the Thr172-containing activation loop of the α-subunit with the β-subunit *via* the linker segment (residues 300−370).[24] In the AMP and, presumably, ADP structures, the latter interaction is stronger, thereby protecting Thr172 from dephosphorylation and associated AMPK deactivation. However, the mechanisms underlying the effects of phosphorylation on the active site performance are currently unclear. Similarly, the mechanism of direct transmission of the allosteric effect from another nucleotide-binding site to the active site remains to be elucidated.

The recently reported full-length structure of a eukaryotic ClC[11] provided a breakthrough in understanding the mechanism of chloride transport and allowed informed speculations on the mechanism of its regulation by nucleotides.[10] The CBS2 domain contacts α-helix D directly and α-helix R indirectly (*via* an α-helix R linker), both of which form the channel pore and selectivity filter. This structure supports and extends earlier findings showing that α-helix R and α-helix R linkers are involved in transmitting conformational changes to the pore.[11,74,75] Interestingly, ClC is also regulated by phosphorylation,[76] but its interplay with the mechanism of nucleotide regulation is unknown. Structural data have been also employed to identify putative signal-conducting structure elements in CBS-PPase.[6]

CONCLUSIONS

CBS domains form a large and widely distributed domain superfamily, displaying an array of different architectures. Typically, four CBS domains from one or two polypeptides form a structural unit that is able to bind adenine nucleotide, metal ion, or unspecific ionic ligands. CBS domains appear to act as allosteric "internal inhibitors". In few documented cases, ligands induce a conformational change in the CBS domain, which is somehow transmitted to functional domains, and either alleviate or promote the inhibition. The binding strength and direction of the effect depend on the ligand identity, making CBS domains useful modules for sensing cellular energy status, metal ion concentration, or ionic strength.

Despite the progress achieved with some proteins and isolated CBS domains, the molecular mechanisms and structural determinants of communication between the CBS domains and functional regions are still unknown. This appears to be the major direction for future studies, with determination of the pairs of full-length structures of active and inactive protein complexes as the major goal. These studies should not, however, overlook the possibility of allosteric regulation through changes in the dynamic behavior of the protein without a concomitant structural change. This noncanonical type of allostery, entropic by nature, has been recently demonstrated for a number of proteins.[77] A related query is the means by which CBS domains exert their autoinhibitory action. The issue may be resolved by comparing the structures and the dynamic behavior of functional proteins with and without the regulatory regions (*i.e.*, full-length and truncated cystathionine β-synthase, CBS-PPase, and its unregulated bacterial homologues). Development of molecules that target CBS domains to either upregulate or downregulate the functions of the proteins containing these modules may provide a

way to combat the hereditary diseases associated with mutations in these regions.[3] Finally, the findings that CBS domains form separate structural units in proteins and their deletion from cystathionine β-synthase does not affect catalytic function suggest an intriguing possibility to use CBS domains as building blocks in the design of regulatable proteins for industrial and other applications.

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ABBREVIATIONS

AdoMet, S-adenosyl methionine; AMPK, adenosine monophosphate-activated protein kinase; AP_4A, diadenosine 5′,5-P1,P4-tetraphosphate; CBS, cystathionine β-synthase; CBS-PPase, CBS domain-containing inorganic pyrophosphatase; ClC, chloride-conducting ion channel; IMPDH, inosine-5′-monophosphate dehydrogenase; MgtE, Mg^{2+} transporter family; OpuA, glycine betaine transporter

KEYWORDS

Adenine nucleotide: compound composed of adenine, ribose, and one to three phosphate groups that has a vital role in nucleic acid synthesis and energy metabolism; Allosteric regulation: regulation of a protein by binding an effector molecule at the allosteric site physically distint from the protein's active site; the allosteric regulation occurs through a conformational change in the protein or a change in its dynamic behavior; AMP-activated protein kinase: an enzyme catalyzing protein phosphorylation by ATP and allosterically regulated by AMP binding to CBS domains that plays an important role in cellular energy homeostasis; Bateman module: a tight structure formed by a pair of tandem CBS domains; CBS domain: a protein domain named after cystathionine β-synthase, one of the proteins it is found in; CBS domains often bind adenine nucleotides and play a regulatory role in proteins; CLC chloride channel: a family of anion-conducting membrane pore proteins, often having a Bateman module in the C-terminal part; Inorganic pyrophosphatase: an enzyme converting pyrophosphate to phosphate; Hereditary disease: a disease that can be passed down through generations of the same family; MgtEa: A bacterial membrane Mg^{2+} transporter with a CBS domain pair in the cytoplasmic region; Protein phosphorylation: adding phosphate covalently to certain residues in proteins; phosphorylation is often used to regulate activities of enzymes and other proteins.

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