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CBSXs are sensor relay proteins sensing adenosine-containing ligands in Arabidopsis

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We recently determined that CBSX proteins, which have only one pair of cystathionine β-synthase (CBS) domains, directly regulate the activation of thioredoxins and thereby control cellular H_2O_2 levels and modulate both plant development and growth. The Arabidopsis genome contains six CBSXs, and these are localized to different subcellular compartments-CBSX1 and CBSX2 in the chloroplast, CBSX3 in the mitochondria, CBSX4 in the cytosol, and CBSX5 and CBSX6 in the endoplasmic reticulum. The CBSXs have been identified in prokaryotes and plants, but not in animals. The considerable differences in length and amino acid sequence between CBSX members may result in variations in protein structure and in their specificity to interact with ligands and/or target proteins. Here, we discuss the possibility that the CBSXs are novel sensor relay proteins that use adenosine-containing molecules as a ligand.

Keywords: CBS domain, CBSX, adenosine-containing ligand, sensor relay, thioredoxin, redox

Abbreviations: CaM, calmodulin; CBL, calcineurin B-like; CIPK, CBL-interacting protein kinase; CBS, cystathionine β-synthase; CBSX, protein having only one pair of CBS domains without any other protein domains; CBSCBS, protein having two pairs of CBS domains; CDCP, CBS domain-containing protein; Trx, thioredoxin; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide; SAM, S-adenosyl methionine

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Due to their sessile nature plants have evolved various inducible defense mechanisms, such as intracellular signal sensor proteins and signaling transduction networks, to overcome many of the environmental challenges they may encounter. One of the more important intracellular signal sensing and transducing mechanisms is sensor relay. Sensor relay proteins have no specific function on their own and possess no intrinsic enzymatic activity; however, following binding to signal molecules, such as ligands or second messengers, they participate in regulating the activity of variable downstream partner proteins in a specific signal cascade. To date, only two types of proteins have been designated sensor relay proteins: calmodulins (CaM) and calcineurin B-like (CBL) proteins. CaMs and CBLs are representative sensor relay proteins; as such, following binding to the second messenger Ca^{2+}, they transduce signals through bimolecular interactions.[1] A total of seven CaMs and ten CBLs have been identified in the Arabidopsis genome. However, based on the amino acid sequence, there are actually only four different CaM proteins; consequently, the seven Arabidopsis CaM proteins are classified into four groups, with all CaM members in the same group having an identical amino acid sequence. The CaMs are able to interact with a wide variety of downstream proteins, including kinases, metabolic enzymes, transcription factors, channel proteins, among others.[2-5] In comparison to CaMs, the CBLs have a relatively more restricted regulating capacity in that they interact with and regulate exclusively the activity of CBL-interacting protein kinases (CIPK). However, CBL3 has recently been reported to interact directly with and regulate the activity of methylthioadenosine nucleosidase, thereby indicating that CBLs may also have a variable regulating ability, similar to CaMs.[6] Despite the wide diversity of regulatory activities shown by CaMs and CBLs, these two sensor relay proteins cannot account for all of the intracellular signal transduction mechanisms that function in plants. Moreover, judging from the multitude of signals initiated by various stimuli and the tremendous complexity of the targets with which the sensor relay proteins interact, it seems reasonable to assume that there may be other

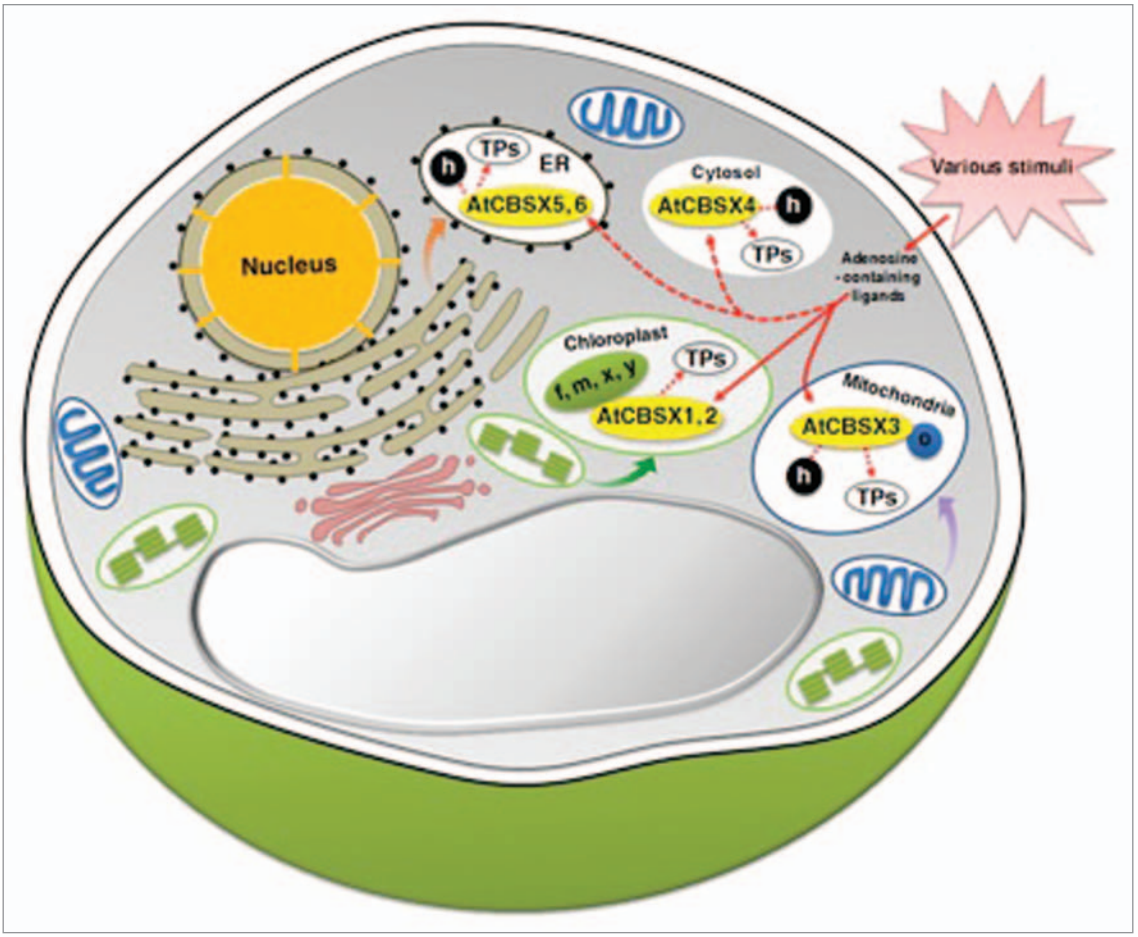

Figure 1. Schematic diagram of proposed function of all CBSX members in the cell. Extracellular stimuli alter the concentration of various adenosine-containing ligands, which in turn is sensed by CBSXs and CBSXs are augmented by binding to a specific adenosine-containing ligand, resulting in a transduction of signals via a sensor relay mechanism. TPs, target proteins; f, Trx f; h, Trx h; m, Trx m; o, Trx o; x, Trx x; y, Trx y.

signal molecules and sensor relay proteins that interact in a manner similar to Ca^{2+} and the calcium sensor relay proteins.

Possible candidate signal molecules/sensor relay proteins are the adenosine-containing metabolites and proteins, denoted CBSXs, which are characterized by containing only one pair of cystathionine β-synthase (CBS) domains—and no other protein domains. A total of six CBSXs have been identified in the Arabidopsis genome.[7] CBSX1 and 2 activate thioredoxins (Trxs) in the chloroplast and in turn helps to regulate cellular redox levels.[8] Those six Arabidopsis CBSX proteins are differentially localized in the subcellular compartments, with CBSX1 and CBSX2 found in the chloroplast, CBSX3 in the mitochondria, CBSX4 in the cytosol, and CBSX5 and CBSX6 in the endoplasmic reticulum. The six different types of Trxs are also differentially distributed throughout the cell, showing a similar subcellular localization as the CBSXs, with types f, m, x and y in the chloroplast, o in the mitochondria, and h in the cytosol, mitochondria, endoplasmic reticulum and extracellular compartment.[9-13] Our group has reported that CBSX3, whose localization is predicted to be in the mitochondria, regulates mitochondrial Trx o. The activities of all of these CBSX members are augmented by binding with adenosine monophosphate (AMP).[8] The reduced Trx has a function in that it is able to reduce the regulatory intramolecular disulfide bonds of target proteins, thereby altering the activity of these target proteins. A great many proteins have been identified as targets of Trxs.[14] These results suggest that CBSXs sense changes in the adenosine-containing ligand (e.g., AMP) in most parts of the cellular compartments and transduce signals through their regulation of the activation of Trxs (**Fig. 1**). As such, this sensor relay protein system resembles the transduction of signals by CBLs through regulating the activities of CIPKs by sensing changes in cellular Ca^{2+} levels.

Bioinformatics studies of the CBSX proteins indicate that, with the exception of CBSX1 and 2, each CBSX that has been identified to date is distinct in terms of length, localization and expression pattern (**Table 1**). In particular, the amino acid similarity of these proteins is apparently quite low among members. These features, namely, variable length and low amino acid homology, suggest that the actual structure of each CBSX member is different. These sequence and structural variations could affect their binding specificity and affinity with adenosine-containing ligands, as well as with their downstream partner proteins,

leading to the notion that this variability of CBSXs in sensing ligands and in binding target proteins broadens the ability of the plant to recognize extracellular stimuli and subsequently to transduce the various signals much more appropriately. This notion is well supported by experimental results. First, CBSX1 has been shown to have a variable ability to interact with downstream partner proteins.[8] Second, the CBS domains recognize their own specific adenosine-containing ligands, such as AMP, adenosine diphosphate (ADP), adenosine triphosphate (ATP), diadenosine polyphosphate, nicotinamide adenine dinucleotide (NADH) and S-adenosyl methionine (SAM).[8,15-19] Third, the CBS domain-only protein in *Methanocaldococcus jannaschii* binds to double-stranded DNA.[20] It may, therefore, safely be assumed that the CBSXs meet the requirements to be a sensor relay protein like CaM and CBL.

Furthermore, to date, CBS domain-containing proteins (CDCPs) have been found in all kingdoms of life form except viruses. For example, eight CBS domain-containing proteins have been identified in *Escherichia coli*, 12 in *Saccharomyces cerevisiae*, 34 in *Arabidopsis thaliana*, 59 in *Oryza sativa* and 75 in *Homo sapiens*.[7,21,22] Interestingly, however, as explained above, proteins comprising only a pair of CBS domains without any other protein domains have been identified in prokaryotes and plants—six in Arabidopsis and 12 in Oryza,[7,22] but as yet no such proteins have been found in animal genomes. Moreover, four and eight proteins having two pairs of CBS domains (CBSCBS) without any other protein domains have been found in Arabidopsis and Oryza, respectively.[7] As previously reported in our article, the CBSXs form a homodimer to achieve their proper function,[8] which implies that these CBSCBS proteins may have similar functional properties with the CBSX proteins. As a consequence, the absence of CBSX and CBSCBS proteins in animals and their presence in prokaryotes and plants strongly imply that due to their sessile nature, plants may have evolved many more perception-response mechanisms than animals to perceive various extracellular stimuli.

Table 1. CBSX proteins in *Arabidopsis thaliana*

Name	Locus	A.A. length	Subcellular localization	Stimuli response
CBSX1	At4g36910	236	Chloroplast	Yes
CBSX2	At4g34120	238	Chloroplast	No
CBSX3	At5g10860	206	Mitochondria	No
CBSX4	At1g80090	402	Cytosol	Yes
CBSX5	At4g27460	391	Endoplasmic reticulum	Yes
CBSX6	At1g65320	425	Endoplasmic reticulum, Vacuole	Yes

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