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Mini Review

Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins

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The cold shock domain (CSD) is among the most ancient and well conserved nucleic acid binding domains from bacteria to higher animals and plants. The CSD facilitates binding to RNA, ssDNA and dsDNA and most functions attributed to cold shock domain proteins are mediated by this nucleic acid binding activity. In prokaryotes, cold shock domain proteins only contain a single CSD and are termed cold shock proteins (Csps). In animal model systems, various auxiliary domains are present in addition to the CSD and are commonly named Y-box proteins. Similar to animal CSPs, plant CSPs contain auxiliary C-terminal domains in addition to their N-terminal CSD. Cold shock domain proteins have been shown to play important roles in development and stress adaptation in wide variety of organisms. In this review, the structure, function and regulation of plant CSPs are compared and contrasted to the characteristics of bacterial and animal CSPs. [BMB reports 2010; 43(1): 1-8]

Structure of cold shock domain proteins

Bacteria encode Csps that are of small size (67-73 amino acids) and consist of a single nucleic acid-binding cold shock domain (CSD) (1, 2). Within the CSD, two consensus RNA binding motifs are present (RNP-1 and RNP-2), which are also present in RRM-type RNA binding proteins (3-5). Studies on the three-dimensional structures of two *E. coli* Csps (CspA and CspB) placed the RNP-1 and RNP-2 motifs on separate juxtaposed adjacent β-strands within the CSD (6-8). A similar structure was also observed for the CspB protein from *Bacillus subtilis* (5). Aromatic residues residing within the RNP-1 and RNP-2 motifs are critical for facilitating ssDNA binding activity by enabling base stacking (7, 9) without apparent sequence specificity. CspB from *Bacillus subtilis* and CspD from *E. coli* purify as dimers in solution. In the case of *B. subtilis* CspB, dimers are formed between two anti-parallel CspB molecules through interactions between the β4-β4 and β4-N terminus (5, 10). On the other hand, CspA from *E. coli* was crystallized as a monomer (6, 7).

Unlike bacterial Csps, their eukaryotic counterparts contain auxiliary domains in addition to a cold shock domain and are often referred to as Y-box Binding proteins (YB). In human YB-1, three structural domains are recognized: a small domain at the N-terminus which is Ala and Pro rich (A/P domain), a central cold shock domain (CSD), and a C-terminal domain (C domain) with alternating clusters of positively and negatively charged amino acid residues (four clusters of each charge) (11, 12). Among bacterial Csps and YB-1, the ~70 residues that comprise the CSD represent the only region that exhibits a high level of sequence conservation. Studies on the CSD of YB-1 revealed a β-barrel spatial structure bearing similarity to bacterial Csps with a similar arrangement of RNA binding motifs (5, 10, 13). The C-terminal auxiliary domain was proposed to have a nonspecific affinity for RNA and DNA which may result from its interaction with negatively charged phosphate groups of nucleic acids (14). It also serves as a docking site for other interacting proteins (15). Similar to bacterial Csps, YB-1 forms large homomultimeric complexes (16, 17). The domain structure among vertebrate Y-box proteins is essentially similar with 100% sequence homology in the CSD. The C-terminal tail domain in vertebrate Y-box proteins is highly divergent and is used to distinguish germ cell and somatic cell type Y-box proteins (18). The three dimensional structure of a plant CSD has not yet been reported. Higher plant CSPs are glycine-rich proteins and are distinct in that they contain two types of nucleic acid-binding modules, a single N-terminal CSD and variable numbers of C-terminal retroviral-like CCHC zinc fingers that are interspersed by glycine-rich regions (see Fig. 1) (19).

Functions in cold stress adaptation

Bacterial Csps play critical roles during the cold adaptation

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Fig. 1. *Comparison of cold shock domain protein structure from bacteria and plants*. Bacterial cold shock proteins are comprised solely of a cold shock domain. Two consensus RNA binding motifs (RNP-1 and RNP-2) reside within the cold shock domain and mediate RNA binding activity. Plant cold shock domain proteins contain an N-terminal cold shock domain and commonly contain auxiliary C-terminal glycine rich regions that are interspersed by a variable number of CCHC retroviral-like zinc fingers. Abbreviations are as follows: cold shock domain (CSD), glycine-rich regions (GLY) and zinc fingers (ZN).

process. *E. coli* contains nine Csps named from CspA to CspI (20), of which only CspA, CspB, CspE, CspG, and CspI are cold inducible (2, 21-23). Upon cold stress, synthesis of the major cold shock domain protein CspA is highly induced, accumulating to more than 10% of the total protein in cold-shocked cells (2). The binding of CspA to RNA is associated with the destabilization of secondary structures in RNA, thereby facilitating translation at low temperatures, where formation of secondary structures in RNA is common (see Fig. 2) (24). Later, an RNA chaperone activity was attributed to CspE protein (25, 26). However, a quadruple deletion of CspA, CspB, CspG, and CspE is required to make the *E. coli* cells cold sensitive (27) indicating the redundancy of function for Csps during exposure to low temperature stress.

Until recently, the cellular functions of vertebrate Y-box proteins were not correlated to low temperature stress. Recently, a downshift in the cell culture temperature of YB-1-depleted chicken cells was shown to lead to growth arrest. Cell growth under low temperature was restored by the expression of an epitope-tagged YB-1 protein in the gene disruptants. This was the first indication that CSD proteins are important for cold adaptation in higher vertebrates (28).

In plants, expression studies on a wheat cold shock domain protein (WCSP1) revealed an up-regulation on both the transcript and protein level during cold acclimation (29). WCSP1 complements the cold sensitive phenotype of the quadruple Csp-deletion strain of *E. coli* (BX04), indicating that this protein can function as an RNA chaperone *in vivo* in a bacterial system (30). Expression studies revealed that some Arabidopsis *CSP* transcripts are also increased upon cold stress treatment, indicating a potential role in cold adaptation (19, 31, 32). **A**rabidopsis **t**haliana **c**old **s**hock domain **p**roteins (AtCSPs) promote cold adaptation in bacteria (31-34). Using functional approaches, AtCSPs were shown to play an important role under low temperature stress conditions *in planta* (33, 34). In rice, a plant which cannot acclimate to cold conditions, cold shock domain proteins were not induced in response to low temperature stress. However, *in vitro* and *in vivo* studies demonstrated that these proteins were capable of functioning in a similar fashion as wheat and Arabidopsis cold shock domain

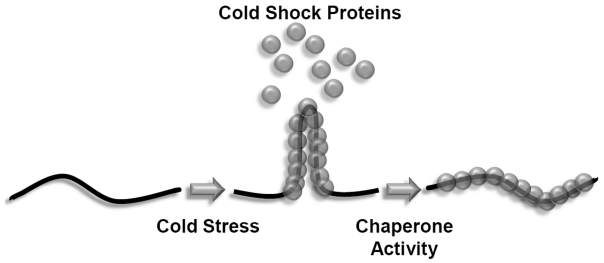

Fig. 2. *Schematic for simplified mode of action for bacterial cold shock proteins*. When exposed to low temperature stress, RNA becomes stabilized and forms secondary structures. Due to the coupling of transcription and translation in bacteria, this can ultimately impede efficiency of translation under low temperature stress. When challenged with low temperature stress, bacteria program a dramatic accumulation of cold shock proteins. Bacterial cold shock proteins function to effectively melt double-stranded regions of RNA transcripts. Secondly, bacterial cold shock proteins function as "chaperones" by maintaining RNA in a single-stranded state, thereby facilitating enhanced translation under low temperature stress.

proteins, indicating a correlative role for cold shock domain proteins in the cold acclimation process (35). Using the extremely freeze tolerant red-osier dogwood model system, protein blot analysis of endogenous CSPs indicated a clear seasonal regulation of CSPs which correlated to periods of maximum levels of freeze tolerance (36). Despite this evidence regarding the relationship of cold shock domain proteins in the adaptation to low temperature stress, their mode of action *in planta* and their importance during cold acclimation remain poorly understood.

Functions in DNA metabolism

Bacterial Csps have been functionally linked to the maintenance of chromosome structure and DNA replication. In *E. coli*, camphor induced chromosome decondensation was reversed by overexpression of CspE and CspC (37). In addition, CspD acts as a novel inhibitor of DNA replication and plays a role in chromosome replication during nutrient stress (38). Similar to prokaryotic Csps, eukaryotic YB-1 plays an important role in various aspects of DNA metabolism. YB-1 moves from the cytoplasm to the nucleus in response to UV light and DNA-damaging chemicals (17, 39). YB-1 exhibits a stronger affinity toward secondary structures in damaged DNA and aids in DNA repair (17, 40). YB-1 plays a role in DNA recombination by promoting complementary DNA strand exchange (40, 41). Similar to bacterial Csps, YB-1 functions in DNA replication (42, 43). Thus, YB-1 appears to be involved in majority of the DNA dependent process. To date, plant CSPs have not been studied in relation to DNA recombination and repair.

Transcription

In *E. coli*, Csps affect transcription by acting as transcription antiterminators. *In vitro* studies indicated that CspE protein inhibited phage lambda Q-mediated transcriptional antitermination (44). Overexpression of the CspE protein resulted in high expression of several promoter-distal genes in the metY-rpsO operon due to transcription antitermination (45) Using *in vitro* and *in vivo* studies, CspA, CspE, and CspC proteins were demonstrated to work as transcription antiterminators at ρ-independent terminators (45). In addition, CspE associates with nascent RNA in transcription elongation complexes implicating a role in transcription (44). The transcription pause efficiency of RNA polymerase was shown to be increased by CspE and CspA protein (46, 47).

The role of human YB-1 has been well studied in relation to transcription. Initially, this protein was identified by its ability to bind to the Y-box sequence (inverted CCAAT motifs) of MHC class II promoters (48). The binding of YB-1 to Y-box sequences influences the transcription of genes either positively or negatively (49, 50). Numerous genes that are important for normal cellular functions are transcriptionally regulated by the YB-1 protein (18). During the G1/S phase transition or in response to thrombin, YB-1 is transferred from the cytoplasm to the nucleus and activates the transcription of certain genes such as cyclin A and B1 (51) or those involved with endothelial cell differentiation (52). In the case of plant CSPs, no evidence has been produced to suggest that they may function to directly affect transcription. However, CSPs from wheat and Arabidopsis are localized to the nucleus (30, 32-34, 53), which indirectly supports a putative functional role related to transcription.

Translation

The majority of *E. coli* Csps are capable of binding RNA (20, 24, 54). Under low temperature stress, a stabilized secondary structure formation in RNA is thermodynamically favored and hinders protein translation. The major cold-induced Csp (CspA) functions as an RNA chaperone and destabilizes secondary structures (24). The CspE protein melts partially double stranded and hairpin structures (26) and was also shown to by bind poly-A tails, thereby stabilizing mRNA by reducing degradation by PNPase and RNaseE (55). In addition to the ability of bacterial cold shock proteins to melt nucleic acids, they also impact mRNA stability. Specifically, CspC and CspE have a dramatic impact on the stabilization of transcripts for a global stress response regulator (rpoS) and the universal stress response protein (uspA) (56). Hence, bacterial Csps function in translation by acting as chaperones and by also affecting the mRNA stability of stress related genes.

The role of YB-1 in translation has been extensively studied. YB-1 protein, known originally as p50, was detected in mRNP (messenger ribonucleoprotein) preparations from various cells and organisms (57, 58). YB-1 exhibits a high affinity for mRNPs and acts as a structural protein in their spatial organization (12, 59). YB-1 exists as a major component of free mRNPs that are not currently being translated, while the polysomal mRNPs, which are being translated, contain poly(A)-binding protein (PABP) along with YB-1 (60-62).

YB-1 accompanies mRNAs throughout their "life cycle" by binding to newly emerging pre-mRNA on chromosomes (49, 63). Depending on the ratio of YB-1 to mRNA, YB-1 differentially affects translation. Translation is stimulated when there is little YB-1 and translation is completely suppressed at a higher YB-1/mRNA ratio (62). The YB-1 mediated regulation of translation occurs only during translation initiation (64, 65). It was proposed that YB-1 stimulates translation initiation by promoting the 43S pre-initiation complex to scan the 5'-untranslated region (5'-UTR) in search for an initiation codon (64). YB-1 inhibits translation by blocking the interaction between mRNA and translation initiation factors, primarily eIF4G, at the first step of initiation (65). Both *in vitro* and *in vivo* studies confirmed that 5′-capped mRNAs are stabilized by YB-1 (65-67). The cold shock domain of YB-1 interacts with the cap and this interaction inhibits the action of cap-cleaving enzymes. Thus, YB-1 promotes the accumulation of mRNAs in the form of free mRNPs for stable storage by suppressing translation and the removal of the 5′ cap (18, 65, 67). In addition to a role in translation initiation, evidence is accumulating that YB-1 plays a role in alternative splicing of pre-mRNA in the cell nucleus (68, 69).

In plants, the role of cold shock domain proteins in the translation process has not been well studied. It is well known that plant CSPs can complement the cold-sensitive phenotype in the *E. coli* CSP quadruple deletion strain (BX04), suggesting that plant CSPs can also act as chaperones during cold stress (30, 31). The sub-cellular localization of WCSP1 to the endoplasmic reticulum indirectly suggests that it may perform a role in translation (30). NAB1, a CSP from Chlamydomonas, stabilizes the mRNA of the chlorophyll binding protein and represses its translation by sequestering it into non-translated mRNPs at the translation pre-initiation stage (70). Taken together, these results suggest a potential role for plant CSPs in translation.

Regulation of cold shock domain gene expression

Among the bacterial Csps, *E. coli CspA* has been most extensively studied in terms of gene expression. The previous notion that CspA is regulated solely by a cold responsive transcription factor (71) was proven incorrect with the discovery that the natural promoter of *CspA* is not required for its cold induction (72, 73). The *CspA* transcript is highly unstable at 37°C and its stability is greatly increased after cold shock (73, 74). Subsequent experiments revealed that the 5′ untranslated region (UTR) of *CspA* transcripts play a critical role in confer-

ring the instability of the transcript (72). The complete deletion of its 5′UTR region resulted in high levels of *CspA* transcripts at 37°C (73). The discovery of potential RNaseE recognition elements in the 5′ UTR led to the hypothesis that *CspA* transcript cleavage by RNaseE at 37°C results in its extreme instability (74). During cold shock, *CspA* transcripts are initially stabilized, however their stability is decreased during cold acclimation due to transcript degradation by the PNPase enzyme (75). In addition, the "cold box", a negative cis-element in the 5′ UTR of the *CspA* transcript, can be recognized by the CspE or CspA protein, thereby increasing the transcription pause recognition (46, 76). Thus *CspA* expression is regulated post-transcriptionally through its 5′UTR.

Similar to *CspA*, expression of the vertebrate YB-1 protein is also regulated on a post-transcriptional level. In contrast to CspA, an important short nucleotide sequence within its 3'-UTR was identified (77). This short sequence was sufficient to suppress the translation of not only YB-1 mRNA but also of other mRNAs in a cell-free translation system. This observation led to the hypothesis that proteins involved in translation initiation are selectively bound to this sequence. Later, UV inducible cross-linking experiments identified YB-1 and PABP as the main proteins that bound to this sequence (77). In a cell free translation system, YB-1 completely suppressed the translation of YB-1 mRNA, while PABP promoted its translation. PABP and YB-1 compete with each other to bind YB-1 mRNA. Specific binding sites of YB-1 and PABP in the 3′UTR were identified using foot-printing (78). The regions overlap each other and contain a common octanucleotide motif, UCCA(G/A)CAA. A comparison of the 3'-UTR nucleotide sequences of mRNAs coding for Y-box proteins of various organisms, from *X. laevis* to humans, revealed the evolutionary conservation of the regulatory region, which is enriched in A and C. These studies suggest that most vertebrate cold shock domain genes are regulated post-transcriptionally.

The regulatory elements and proteins involved in the regulation of plant CSP gene expression are not well characterized at this time. However, comparative expression analysis between two *Arabidopsis thaliana* ecotypes revealed 1000-fold reduction in the expression for AtCSP4/AtGRP2b (At2g21060) in the Landsberg ecotype. Comparative sequence analysis revealed multiple discrepancies in cis-element regions which may impart transcriptional control over this gene locus (79). Using transgenic overexpression lines for the well characterized abiotic stress related CBF/DREB transcription factors, we confirmed that AtCSP3 (At2g17870), which is a regulator of cold acclimation (33), is targeted by the CBF3/DREB1A transcription factor (unpublished results).

Role in development

The quadruple Csp deletion mutants of *E. coli* showed elongated and filamentous cells at 15°C due to defects in cell septum formation (27, 31). The expression of CspD is dramatically induced during stationary phase growth. However, the role of this protein in bacterial development is not clearly understood (38). In higher eukaryotes, cold shock domain proteins play an important role in development by affecting the translation of certain mRNAs during specific developmental stages. For example, YB-1 plays a role in the stable storage of specific mRNAs by binding to them and repressing their translation until needed. mRNA storage is especially important during certain stages of development, during which cells are rapidly dividing and transcriptional activity is minimal (80). For example, the FRGY2 cold shock domain protein is abundant *in Xenopus* oocytes and binds to cytoplasmic maternal mRNAs masking their translation (81). Similar to the situation observed *in Xenopus*, Y-box proteins like MSY1, MSY2a, MSY2b, and MSY4, have been identified in the germ cells of mice (82-84). MSY2 accounts for ~2% of total oocyte protein and is present in early and mature oocytes but completely disappears at the late two-cell stage embryo stage, suggesting that MSY2 stabilizes and/or regulates the translation of maternal mRNAs (85). Reduction of MSY2 levels in mouse oocytes results in reduced fertility (86). A high abundance of MSY2 is also observed in meiotic and post-meiotic germ cells in testes (82). MSY2 preferentially binds to mRNAs that are transcribed from a Y-box promoter, thereby linking transcription with translational delay in male germ cells. A lack of MSY2 results in spermatogenic arrest and male infertility (87). In *C. elegans*, the Lin-28 cold shock domain protein controls developmental transitions during early stages (88). Collectively, these results suggest a key role for cold shock domain proteins during development by affecting translation.

Using *Arabidopsis thaliana* as a model plant, its endogenous CSPs are shown to be important during plant growth and development. When the expression of AtCSP2/AtGRP2/CSDP2 (At4g38680) was up- or down regulated, many developmental abnormalities with respect to flowering time, apical dominance and seed development were observed. Furthermore, the transcripts of this gene are abundant in meristematic areas in which rapid cell divisions occur suggesting this protein may function in mRNA storage (53). A subsequent extensive study characterized the expression of the entire Arabidopsis CSP family in relation to stages of floral and silique development (89). A prominent effect of CSPs has also been noted on seed germination during abiotic stress conditions such as dehydration and salt stress (34). In Chlamydomonas, NAB1 aids in the acclimation to high light conditions by regulating the size of light harvesting antenna of PSII by affecting the mRNA stability of light harvesting chlorophyll binding protein (70).

Post-translational modifications of cold shock domain proteins

Unlike bacterial Csps, several vertebrate cold shock domain proteins are modified on the post-translational level. Xenopus FRGY1 and FRGY2 are phosphorylated by casein kinase II and

this modification promotes their capability for binding mRNA, which in turn may affect mRNA silencing during oogenesis (11, 90). For human YB-1, casein kinase II mediated phosphorylation did not affect its RNA binding ability (41). Human YB-1 can also be phosphorylated by AKT kinase which decreases its ability to bind mRNA cap regions. Phosphorylated YB-1 minimizes cap-dependent translational repression and results in the activation of silenced mRNA species (91). PIPPin, which is another vertebrate cold shock domain protein, is SUMOylated in rat brain tissue (92). The functional significance of SUMOylation for this protein is not yet fully understood.

Four Arabidopsis CSPs were recently confirmed to undergo phosphorylation using cell extracts from plants grown under standard conditions (Karlson *et al*., 2009, Thompson *et al*. unpublished results). Bioinformatics and phylogenetic analyses confirmed that phosphorylation sites are highly conserved in plant CSPs from single cell photosynthetic organisms through higher plants. Predictive analysis for conserved SUMOylation signature motifs from lower plants to higher plants identified conserved SUMO sites (Thompson *et al*. unpublished results). SUMOylation for two rice CSPs (36) and Arabidopsis AtCSP1 (Thompson *et al*. unpublished results) has been confirmed using *in vitro* assays.

Conclusions

In summary, CSPs from bacteria and higher vertebrates mediate various cellular processes by binding to nucleic acids. In bacteria and higher mammalian systems, these proteins function in transcription, translation and DNA-dependent processes like recombination and repair. For plant CSPs, these areas have not yet been tested and will be important areas of future investigation. Similar to bacterial CSPs, evidence is mounting that plant CSPs play an important role in cold stress adaptation. However, a detailed understanding for the precise mode of action relating to low temperature stress still remains to be elucidated.

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