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Review

Correlating multi-functional role of cold shock domain proteins with intrinsically disordered regions

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ABSTRACT

Cold shock proteins (CSPs) are an ancient and conserved family of proteins. They are renowned for their role in response to low-temperature stress in bacteria and nucleic acid binding activities. In prokaryotes, cold and non-cold inducible CSPs are involved in various cellular and metabolic processes such as growth and development, osmotic oxidation, starvation, stress tolerance, and host cell invasion. In prokaryotes, cold shock condition reduces cell transcription and translation efficiency. Eukaryotic cold shock domain (CSD) proteins are evolved form of prokaryotic CSPs where CSD is flanked by N- and C-terminal domains. Eukaryotic CSPs are multi-functional proteins. CSPs also act as nucleic acid chaperons by preventing the formation of secondary structures in mRNA at low temperatures. In human, CSD proteins play a crucial role in the progression of breast cancer, colon cancer, lung cancer, and Alzheimer's disease. A well-defined three-dimensional structure of intrinsically disordered regions of CSPs family members is still undetermined. In this article, intrinsic disorder regions of CSPs have been explored systematically to understand the pleiotropic role of the cold shock family of proteins.

1. Introduction

Cold shock proteins (CSPs) are named so due to their key role during response to cold stress in prokaryotes. However, all prokaryotic CSPs are not involved in response to cold stress as they participate in many other cellular and molecular functions. Their eukaryotic homologs have one or more cold shock domains, which is highly similar to prokaryotic CSPs, flanked by N- and C-terminal domains. In human, regulation of CSPs has been extensively studied and several roles are reported for cell functioning, regulation, and maintenance. CARHSP1 binds to and stabilizes tumor necrosis factor (TNF) [1]. Human Y-box binding protein-1 (YB-1) is a cold shock domain protein which is involved in a wide range of biological activities ranging from regulation of transcription, splicing, and translation, to exosomal RNA content modulation [2]. YB-1 is crucial for embryonic development and survival [3]. UNR, another CSP, maintains the pluripotent state of embryonic stem cells [4]. Moreover, CSP's expression patterns have also been linked with cancer and inflammatory diseases [5].Cold exposure increases norepinephrine levels, which promotes focus, vigilance, attention, and mood [6] as well as helps in reducing inflammation in arthritis [7] Cold exposure increases adiponectin level in cell, which helps in blood sugar regulation [8]. Low temperature activates brown adipose tissue, which improves mitochondrial functioning, metabolism, and thermoregulation [9]. Immersion in modestly cold water lowers heart rate, cortisol, systolic blood pressure, and inflammation. Winter swimming lowers uric acid and increases glutathione, which promotes detoxification and antioxidant

Abbreviations: CSD, Cold shock domain; CSPs, Cold shock proteins; YB1, Y-box binding protein-1; CARHSP1, Calcium Regulated Heat Stable Protein 1; CHSP1, Calcium-regulated heat-stable protein 1; PIP, Prolactin-induced protein; G3BP1, Ras GTPase-activating protein-binding protein 1; IDPs, Internally displaced persons; FASTpp, Fast parallel proteolysis; NMR, Nuclear Magnetic Resonance.

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system [10]. Immersion in 14 °C water for one hour increases metabolic rate by 350 %, norepinephrine level by 530 %, and dopamine level by 250 % [11]. Different cold shock proteins in animal, plant, human, and bacteria are summarised in Table 1.

1.1. Bacterial cold-shock proteins

CSPs are highly conserved proteins found in single-cell bacteria and multicellular organisms including plants, animals, and humans. The molecular weight of bacterial CSPs is in the range of 50–70 kDa. In *E. coli,* induction of CspA occurs during the lag period after decreasing the temperature from 37 °C to 10 °C and finally cell growth is resumed [12–16]. The 7.4 kDa CspA protein consists of 70 amino acid residues [17]. Five anti-parallel β-sheets forming a β-barrel structure comprise the protein. RNP1 (Phe18-Gly19-Phe20) and RNP2 (Phe31-Val32-His33) are the two RNA binding motifs present in β-2-sheet and β-3-sheet, respectively [18]. CspA works as RNA chaperone to inhibit the secondary structure formation in RNA molecules at low temperatures [18]. The CspA, CspB, CspE, CspG, and CspI are the only cold-inducible proteins out of nine reported CSPs in *E. coli* [19].

Table 1
List of Cold shock protein in Animal, plant, human, fungal and bacteria with their accession id, common name and gene name. This result Predicted by MobiDB database (URL: https://mobidb.org/).

Accession	Name	Organism	Gene name
Q94C69	Cold shock domain-containing protein 3	*Arabidopsis thaliana* (Mouse-ear cress)	CSP3
Q38896	Cold shock domain-containing protein 4	*Arabidopsis thaliana* (Mouse-ear cress)	CSP4
Q63430	Cold shock domain-containing protein C2	*Rattus norvegicus* (Rat)	CSDC2
Q9Y534	Cold shock domain-containing protein C2	*Homo sapiens* (Human)	CSDC2
O75534	Cold shock domain-containing protein E1	*Homo sapiens* (Human)	CSDE1
Q91W50	Cold shock domain-containing protein E1	*Mus musculus* (Mouse)	CSDE1
P18395	Cold shock domain-containing protein E1	*Rattus norvegicus* (Rat)	CSDE1
O65639	Cold shock protein 1	*Arabidopsis thaliana* (Mouse-ear cress)	CSP1
Q41188	Cold shock protein 2	*Arabidopsis thaliana* (Mouse-ear cress)	CSP2
P0A9X9	Cold shock protein CspA	*Escherichia coli* (strain K12)	CSPA
P32081	Cold shock protein CspB	*Bacillus subtilis* (strain 168)	CSPB
P10863	Cold shock-induced protein TIR1	*Saccharomyces cerevisiae* (strain ATCC 204508/S288c) (Baker's yeast)	TIR1
P33890	Cold shock-induced protein TIR2	*Saccharomyces cerevisiae* (strain ATCC 204508/S288c) (Baker's yeast)	TIR2
O54310	Cold shock-like protein	*Thermotoga maritima* (strain ATCC 43589/MSB8/DSM 3109/JCM 10099)	CSP
P36995	Cold shock-like protein CspB	*Escherichia coli* (strain K12)	CSPB
P0A9Y6	Cold shock-like protein CspC	*Escherichia coli* (strain K12)	CSPC
P0A968	Cold shock-like protein CspD	*Escherichia coli* (strain K12)	CSPD
P0A972	Cold shock-like protein CspE	*Escherichia coli* (strain K12)	CSPE
P0A978	Cold shock-like protein CspG	*Escherichia coli* (strain K12)	CSPG
P0A355	Cold shock-like protein CspLA	*Listeria monocytogenes serovar* 1/2a (strain ATCC BAA-679/EGD-e)	CSPLA
P60824	Cold-inducible RNA-binding protein	*Mus musculus* (Mouse)	CIRBP
P46525	Cold-shock protein CS120	*Triticum aestivum* (Wheat)	CS120

1.2. Plant cold shock proteins

In plants, CSPs have a pleiotropic role in several molecular functions [20]. It is known that many species have one or more than one member of the CSP family. One CSD member, i.e. WCSP1, is found in wheat that is up-regulated in cold, and plays key role in cold acclimation [21]. Two CSP family members are found in rice which are involved in cold stress adaptation [22]. Four CSP proteins are present in *Arabidopsis thaliana* and three of them are found to provide freeze tolerance along with other functions [23–26]. Chickpea has one CSD protein which exhibits single-stranded nucleic acid-binding activity [27]. In most of the plant species, the CSD proteins are involved in several functions viz. seed germination, plant development, flowering, embryo development, silique development, along with cold acclimation [20]. The C-terminal domains of CSPs in plants are rich in glycine residues. Three dimensional structure predictions of these regions result in loop formation, suggesting the lack of globular or fixed structure in the region. While bacterial CSPs and plant CSP's CSD share fair similarities, the plant and animal CSPs both have low complexity regions present in the C-terminal domains.

1.3. Animal cold shock proteins

Majority of the animal genomes possess genes coding for members of the CSD protein family. CSDs are reported in various eukaryotic proteins since their discovery in bacterial CSPs. CSDs are generally embedded in larger proteins of multicellular organisms [28]. The *YBX1* protein (a cis-acting component) specially binds to the Y-box which bind on HLA class-II gene's upturned CCAAT box (ATTGG) and regulate their essential expression. Studies suggest that *levels* of *HLA-DRβ* chain mRNA, a beta chain of antigen-presenting major histocompatibility complex class II (MHCII) molecule, and *YBX1* expression are inversely correlated [29].

The hydrophilic C-terminal domain of frog Y-box protein 2 (FRGY2) interacts with RNA independent of the CSD but this binding is sequence specificity independent. Therefore, FRGY2 protein exhibits sequence-specific and non-specific RNA binding by CSD and C-terminal domains respectively [30]. An alignment of animal CSD protein sequences suggested that they share high similarity and are prevalence of conserved regions across animal taxa [31]. In zebrafish, *YBX1* interacts with m5C-modified target mRNA by binding with W45 residue and facilitates early embryogenesis [32]. Drosophila msl2 gene (male-specific lethal-2) mediates the gene-dosage reparation between male and female flies. CSDE1 and SXL (Sex-lethal) genes down-regulate the translation of msl2 mRNA, and both of them bind at the msl2 3′ UTR region [33].

Lin28 contains two nucleic acid interacting domains, a cold shock domain and two tandem Cys-Cys-His-Cys (CCHC)-type zinc-binding motifs (CCHCx2). LIN28A interacts with preElet-7 microRNA, miRNAs that control many cell-fate determination genes. The study reports that the LIN28A cold shock domain has ability to accommodate rather diverse structures with stem-loop [34]. The m^5C-modified mRNAs are recognized by Y-box binding protein 1 (*YBX1*) in zebrafish via π-π interactions at Trp45 which is a key amino acid in the *YBX1* cold shock domain. This underlines the role of CSD proteins in early embryogenesis and maternal mRNA stability [35]. In Drosophila, maintenance of GSC (germline stem cell), differentiation, and proliferation in the ovary is promoted by YPS (Ypsilon schachtel), a human *YBX1* homolog, by binding to m^5C-containing RNAs [36].

1.4. Human cold shock domain proteins

In human, CSDs are present mainly in YBX1, YBX2, YBX3, Lin28 A,

Lin28 B, CARHSP1, PIP protein, and UNR/CSDE1 proteins [5]. In mouse, the YBX1 knockouts are embryonic lethal [3]. YBX1 has key role in the colorectal, lung, and breast cancer regulation and proliferation [37,38]. The DbpA (DNA binding protein A) and DbpC (DNA binding protein C), two additional family members of *YBX1*, are encoded with genes *YBX3* and *YBX2*, respectively. While expression of *YBX2* is limited to germ cells [39], *YBX1* and *YBX3* are expressed in all tissues at the time of development. Though, after birth, the expression of *YBX3* (DbpA) is considerably down-regulated in most tissues except heart, skeletal muscle, blood vessels, and testis tissues [40,41]. The DbpA_a and DbpA_b, the two isoforms of DbpA, vary by an otherwise spliced exon which codes for a 69 amino acids long distinctive domain positioned contiguous to the cold shock domain [42,43]. The Lin28 is another important CSP expressed in humans that was first characterized in *C. elegans* as a developmental factor [44]. Nevertheless, for cellular reprogramming four factors namely OCT4, SOX2, NANOG, and LIN28 were able to reprogram human somatic cells to pluripotent stem cells. Lin28 is also competent to relapse discerned cells into their pluripotent state [45].

Along with CSD, Lin28A/B also has two distinct CCHC type zinc fingers, which create a knuckle domain that contributes to nucleic acid binding [46]. In this context, the capability of Lin28 inhibits let-7 miRNAs is worth mentioning [46,47]. Lin28 is targeted by let-7 forming a double-negative feedback loop [48]. Lin28 also binds to mRNAs to regulate translation, partaking in many ribonucleoprotein complexes, like stress granules and P-bodies [49]. CARHSP1 (Calcium-regulated heat-stable protein 1) belongs to human CSP families and it is also recognized as CRHSP-24 (Ca2+-regulated heat-stable protein of 24 kDa) which has 24 kDa molecular weight.

Calcium-regulated heat-stable protein 1 (CARHSP1), initially recognized as calcium/calmodulin-regulated protein phosphatase calcineurin substrate [50], is a brain-specific CSP [51]. CARHSP1 binds to and stabilize TNF mRNA [51]. The expression of PIPPin, another cold shock domain protein, is limited to the brain, where it binds on mRNA and regulates translation [52–56]. Generally, CSPs have units of ribonucleoprotein (RNP) motifs that facilitates RNA binding of the protein. The PIPPin is found to be present with ribonucleoprotein complexes and acts together with hnRNP A1, hnRNP K, and YB-1 (other RNA binding proteins) [57]. This family's another member is located upstream of *NRAS* (UNR) [58,59]. Furthermore, a PIPPin paralog, CHSP1 was colocalized with an initiator of the formation of stress granule G3BP1 [1,60,61]. Initially, *NRAS* gene was known as a *NRAS* expression regulator [62–65]. It was revealed later that UNR codes for a protein having five CSDs that undergo alternative splicing (Fig. 1) [66–68]. Later the gene was renamed as CSDE1. UNR/CSDE1 binds with single stranded DNA or RNA like other CSPs [66,69,70]. For regulating translation and mRNA stability, UNR works along with the PTB (polypyrimidine-tract-binding protein) [71,72]. Like *YBX1*, studies on Unr knockout mice revealed that it is necessary for development. In embryonic stem cells pluripotent state is maintained by Unr [4,73]. Domain architecture of cold shock protein in different organisms is shown in Fig. 2.

1.5. Intrinsic disorders and structural flexibility

Intrinsically disordered proteins (IDPs) lack a stable or systematic three-dimensional structure [75–77], usually in the absence of interacting partners like DNA, RNA or proteins. An intrinsically disordered protein's structure varies from completely unstructured to partly structured entity which comprises secondary or tertiary structures like random coils, molten globule-like aggregates, or linkers in large-sized proteins. Sometimes, IDPs are classified as distinct protein classes [78]. Requirement of a fixed three-dimensional structures of proteins to accomplish the biological functions has been refuted after discovery of IDPs. Many reports about proteins structural dynamics suggest that flexibility contributes to multi-functionality, the rigid protein structures dogma is now abandoned. IDPs, despite the lacking stable structures, are a big and functionally significant proteins class. After binding to other macromolecules, a firm three-dimensional structure can be adopted by IDPs. Overall, IDPs tend to keep characteristically distinct features in

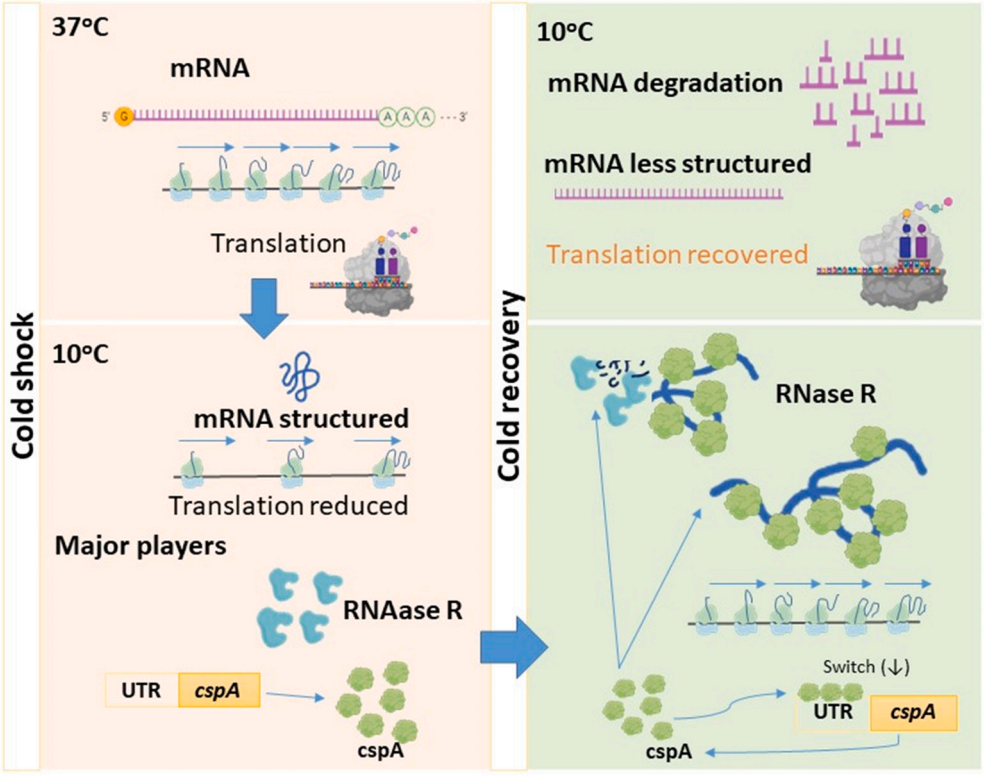

Fig. 1. Theoretical framework of the molecular mechanism of cold shock protein at low temperature.

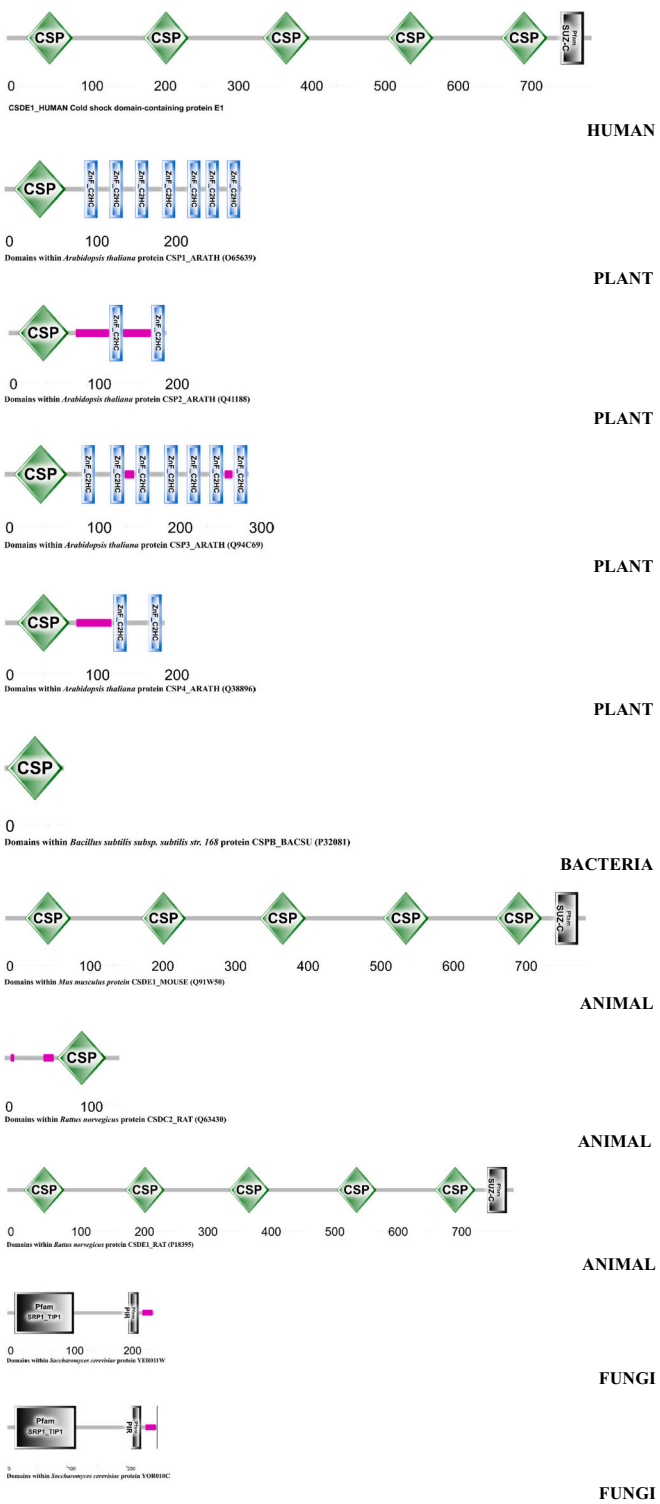

Fig. 2. Domain architecture of cold shock protein in bacteria, plants, animals, fungi, and humans. The domain structure of cold shock protein with length of each protein. SMART (Simple Modular Architecture Research Tool) webserver was used to construct the domain structure of CSPs. (http://smart.embl.de) [74].

terms of function, structure, interactions, sequence, regulation, and evolution. In many ways, they are different from structured proteins [79].

Protein's existence as an assembly of related structures and occurrence of relatively extra restrained regions within them is a well-known fact [80,81]. IDPs harness this flexibility spectrum as they comprise of substantial local structural propensity or supple multi-domain assemblages. In proteins, exceptionally flexible and disordered regions contribute to allosteric regulation and enzyme catalysis, that are functionally essential phenomena [81]. It has been suggested that disordered protein's structural flexibility provides conformational diversity to bind with the modifying enzymes (kinases and phosphatases) in addition to their receptors because many disordered proteins show post-translational binding affinity with their receptors [82]. Disordered regions frequently exist as loops or flexible linkers connecting the domains. Length of linker sequences may greatly vary but linker sequences are characteristically opulent in polar uncharged amino acids. Through protein domain dynamics, the flexible linkers allow the connecting domains to bend generously for binding with other macromolecules. The flexible linkers also enable binding partners to induce greater scale conformational changes in the protein [75,81]. Many roles of short disordered protein segments mediating functional interactions with other RNA, DNA and sugars, are reported. They have been linked with cell regulation, including, cell shape control, protein subcellular localization, and turnover of the regulated proteins [83]. For specific interactions, the affinity of individual linear motifs is often tuned by post-translational modifications. IDPs do not contain spatially disposed of active pockets, unlike globular proteins [84]. Nevertheless, there are linear or pre-structured motifs in 80 % of intrinsically disordered proteins that are temporary secondary structural components seasoned for target recognition [83]. These transient structures are converted to incomplete and completely firm secondary structures, like helices, upon target binding. Thus, pre-structured motifs are the putative active sites in IDPs [83].

Structural alignment and multiple sequence alignment of *E.coli* with human YB-1 (PDB ID: 1H95) and its homologs in *Homo sapiens* (PDB ID: 1H95), *Mus musculus* (PDB ID: 3TRZ), *Corynebacterium pseudotuberculosis* (PDB ID: 5O6F), *Escherichia coli* (PDB ID: 1MJC), *and Salmonella enterica* (PDB ID: 3I2Z) species was performed and it was found that most of the residues that participate in RNA/DNA binding are conserved and share similarity with *E.coli* cold shock proteins. Analysis was done using Jalview Version: 2.11.2.2 [85] (Figs. 3a and 3b).

Several unstructured proteins go through transitions to transform into more ordered states [86]. The coupled binding and folding may be local or encompass a whole protein domain. In regulating specific biological functions, some disordered regions work as “molecular switches” by moving to organized confirmation upon molecular recognition such as DNA/RNA binding, small molecule-binding, and ion interactions, etc. [87]. Conformational freedom of intrinsically disordered proteins can be retained even when intrinsically disordered proteins bind specially to other proteins. In a bound state, the structural disorder can be static or dynamic. The modulation of the conformational ensemble of the complex takes place through protein interactions or post-translational alterations [88]. The specificity of DNA binding proteins frequently hinges on fuzzy fractions, which differ by alternative RNA splicing [89]. Some fuzzy complexes may exhibit high binding affinity [90], although, for the similar system, diverse affinity values have also been reported [91].

According to the cellular conditions, IDPs adopt various structures in vivo, producing a conformational or structural ensemble [3,37]. Hence, conformational changes are highly related to the execution of the biological function. However, in their native state, only few proteins are found to be completely disordered. The disorder is commonly found within the protein, which may also have some well-structured regions. Therefore, the term intrinsically disordered protein comprises proteins that have inherently disordered areas in addition to completely disordered proteins. Amino acid sequence decides the order and quantum of intrinsic disorders [75]. Generally, intrinsically disordered proteins are characterized by occurrence of regions lacking hydrophobic amino acids and having a great polar as well as charged amino acids proportion. [92]. High tendency for interaction with water is observed due to such sequence property in the proteins. The disorder is promoted by high net charges caused by electrostatic repulsions generated from similarly

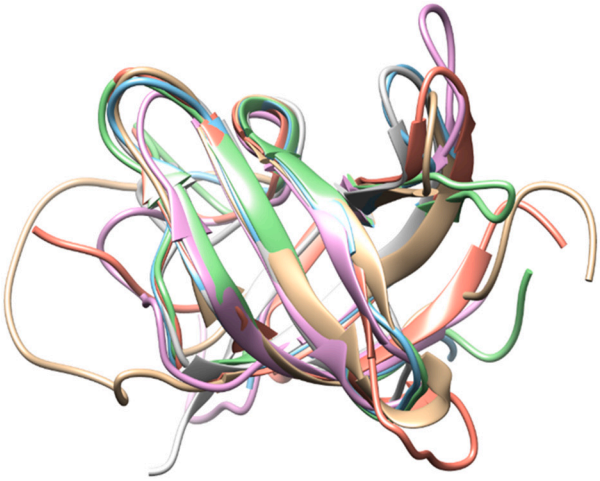

Fig. 3a. : Structural alignment of CSP proteins, Single-stranded DNA-binding cold shock domain (CSD) of human y-box protein 1 (YB1) (1 h95), Crystal structure of CSPA, the major cold shock protein of *Escherichia coli* (1MJC), Solution structure of the first cold-shock domain of the human kiaa0885 protein (UNR protein) (1WFQ), Structure of cold shock protein from *Salmonella typhimurium* (3I2Z), Cold shock proteins (CSPS) (5O6F). Color of structure are same as PDB ID color given in with sequences.

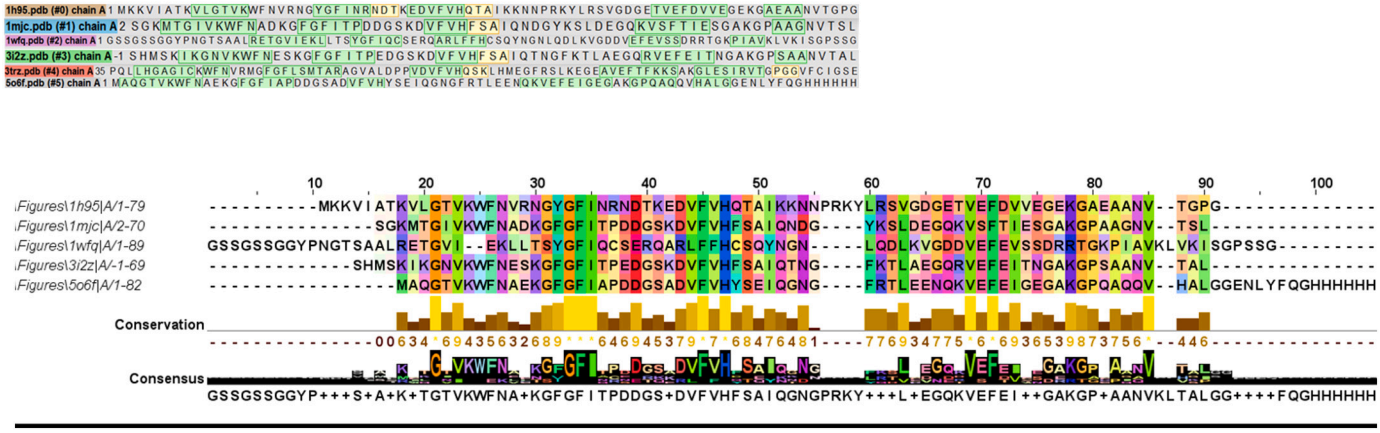

Fig. 3b. The sequence alignment of cold shock proteins by using Clustal Omega (http://www.clustal.org/omega/). Jalview of Clustal omega showing consensus sequence of the highly conserved domain of cold shock proteins. Multiple sequence alignment of Single-stranded DNA-binding cold shock domain (CSD) of human y-box protein 1 (YB1) (1 h95), Crystal structure of CSPA, the major cold shock protein of *Escherichia coli* (1MJC), Solution structure of the first cold-shock domain of the human kiaa0885 protein (UNR protein) (1WFQ), Structure of cold shock protein from *Salmonella typhimurium* (3I2Z), and Cold shock proteins (CSPS) (5O6F). Conserved amino acids (shown by asterisks) are highlighted with different colors depending on whether or not these amino acids coincide with the conserved positions in the consensus sequence. At 21th, 33-35th, 69th, 71th, and 85th position amino acids were found highly conserved (100 %) score.

charged residues [93].

Thus, a hydrophobic core can't be sufficiently buried by disordered sequences to form a stable globular structure. In a few cases, for identifying such regions, evidences from the hydrophobic clusters in disordered sequences suggest that they undergo binding and coupled folding. Regions without any regular secondary structure are reported in several disordered proteins and such regions are termed as flexible, as they fall in the category of loops. The globular structures are rigid and have single fixed Ramachandran angles while multiple sets of angles are involved in the case of intrinsically disordered proteins [93]. Well-structured proteins also have certain level of flexibility but in reference to disordered proteins, flexibility is a different phenomenon. [93]. Low complexity regions, over-populated with a few residues, also contribute to disorders in several proteins. Low complexity sequences are signals of the presence of disordered regions, but all disordered proteins do not necessarily need to have low complexity sequences.

1.6. Intrinsic disorders in cold shock domain proteins

Eukaryotic CSD proteins are multi-domain proteins containing a CSD flanked by C-terminal and N-terminal domains. In human YB1, only the cold shock domain (CSD) has a compact globular structure. The YB1 was included in the list of unreported proteins containing intrinsic disorders [94]. Using solution NMR, the 3D structure of human Y box proteins CSD was first determined by Kloks et al. [95] and later, Yang et al. established RNA-YB1 interaction by high-resolution crystal structures of the YB-1 CSD in complex with different RNA oligomers at 1.7 Å resolution [32]. By using NMR spectroscopy, it has been reported that YB-1 CSD has binding affinity for DNA as well as RNA [96]. The full structure of human YB1 protein was predicted by Birendra et al., and they found that only CSD had a globular structure while other regions in the protein were rich in flexible loops [31]. Mani and Gupta (2015) reported that Plant CSPs also lack compact structure in C- terminal region [97]. The flanking regions in CSPs lack fixed structure, they are generally rich flexible secondary structures like loops, and they are least conserved regions in the protein. The presence of functional regions without a well-defined 3-D structure suggests their role as molecular recognition elements. They act as solubilizers or help in locally loosening the structure of the kinetically trapped folding intermediate via transient binding to facilitate its conformational search [98].

Analysis of the prediction of intrinsic disorders by iupred2a server [99] in Human YB-1 suggests that the conserved cold shock domain is present in the least disordered region. In contrast, N- and C-terminal domains are likely to fall in the disordered regions. In predictions for other eukaryotes, a similar pattern is observed (Fig. 4).

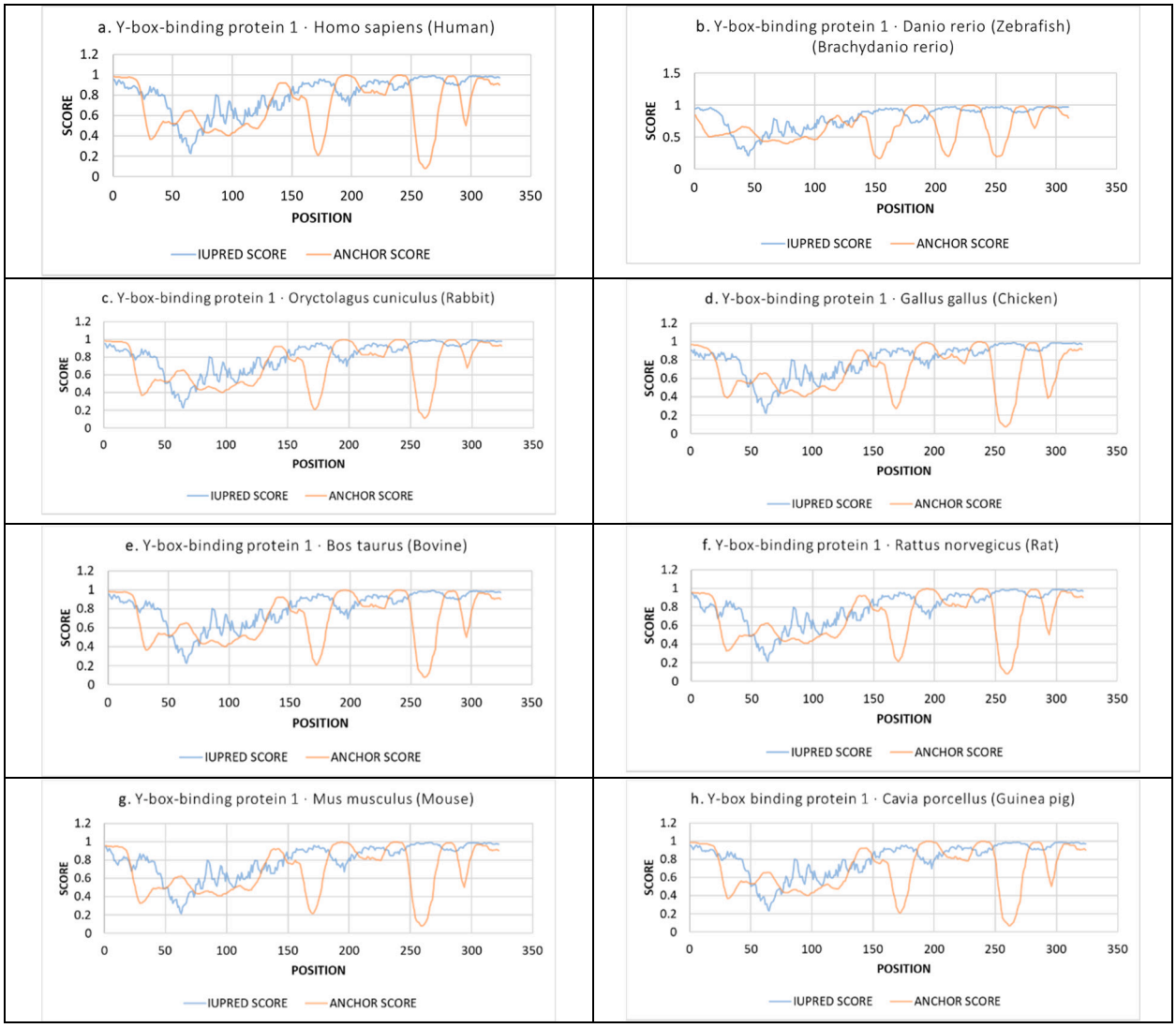

Fig. 4. Prediction of Intrinsic disorders and binding regions of Y-box-binding protein 1 in different species. The output of IUPred2 and ANCHOR2 (cut-off 0.5) for the different species. Scores are shown in orange and blue color. (a) *Homo sapiens* (Human) (b) *Danio rerio* (Zebrafish) (*Brachydanio rerio*) (c) *Oryctolagus cuniculus* (Rabbit) (d) *Gallus gallus* (Chicken) (e) *Bos taurus* (Bovine) (f) *Rattus norvegicus* (Rat) (g) *Mus musculus* (Mouse) (h) *Cavia porcellus* (Guinea pig). (For interpretation of the references to color in this ffgure legend, the reader is referred to the web version of this article.)

1.7. Cold shock domain proteins and pleiotropy

CSPs are multi-function proteins and they interact with different types of biomolecules, including DNA, RNA, as well as proteins. They play key role in transcriptional regulation and post-translation modifications related to several metabolic pathways. Eukaryotic cold shock domain proteins are considered to be highly versatile regulators of gene expression [100]. There are sufficient evidences that CSPs are rich in regions with disordered regions that lack a fixed three-dimensional structure in physiological conditions. Thus the protein falls into a group of intrinsically disordered proteins that do not follow the classical rule 'one protein–one function' but introduce a novel principle stating that a disordered structure suggests many functions. YB-1 participates in a wide variety of DNA/RNA-dependent events, including DNA repair, pre-mRNA transcription and splicing, mRNA packaging, and regulation of mRNA stability and translation. At the cellular level, various activities of YB-1 are manifested by their involvement in cell proliferation and differentiation, stress response, and malignant cell transformation [101].

Human YB-1 specifically binds to Y-box, or with its similar sequences, and with other transcription factors(TFs). It acts either as co-activator or co-repressor. It's binds to the single-stranded region of the promoter to trigger or inhibit the DNA binding of TFs. Transcriptional and *translational regulation* control by YB-1 is generally achieved via RNA-DNA binding activity. During YB-1 deficit conditions translations and degradation is facilitated, as the cap-binding factor is free to bind with mRNA mGppp Ncap and thus mRNA becomes available for translation. YB-1 can regulate translational activity by protein-protein interaction too. Under iron-deficient conditions IRP2 (Iron regulatory protein 2) binds to the 5' UTR of ferritin mRNA and inhibits translation. While under iron-rich conditions, iron binds to IRP2 protein and forms a complex with YB-1. This interaction leads to the release of these complexes from mRNA which makes mRNA accessible for translation. YB-1 is mainly localized in the cytoplasm and interacts with RNAs and cytoplasmic proteins. UV irradiation, Chemotherapy, and other stimuli can initiate nuclear translocation of YB-1. However, YB-1 in complex with other proteins has also been reported in many cellular functions [102].

CSPs belong to a highly conserved family of proteins and they are present in prokaryotes, animals, and plants. CSPs share a sufficient amount of similarity at the sequence level despite of having different functions across taxa. Their functional versatility depends on the structural flexibility provided by a combination of fixed and disordered

regions present in their domains. Though, the crystal structures of full length prokaryotic cold shock proteins have been determined but they CSPs are known to have limited roles in cellular and molecular functions in comparison to their eukaryotic counterparts. The structures of full length eukaryotic cold shock domain proteins are not determined so far. Crystal Structures of YB-1 and other cold shock domain protein are available but they are only limited to the cold shock domains [103–105]. The structures of eukaryotic N terminal and C terminal domains of CSPs have not been determined so far, most possibly due to their structural flexibility as structure determination via crystallography or NMR is challenging for proteins where localized regions or even their entire structure fail to fold into a three-dimensional form [106].

1.8. Molecular recognition features (MoRFs)

IDR binding sites are categorized as: Short Linear motifs (SLiMs) and molecular recognition features or elements (MoRFs). Short segments within longer disordered protein regions that have experimentally been

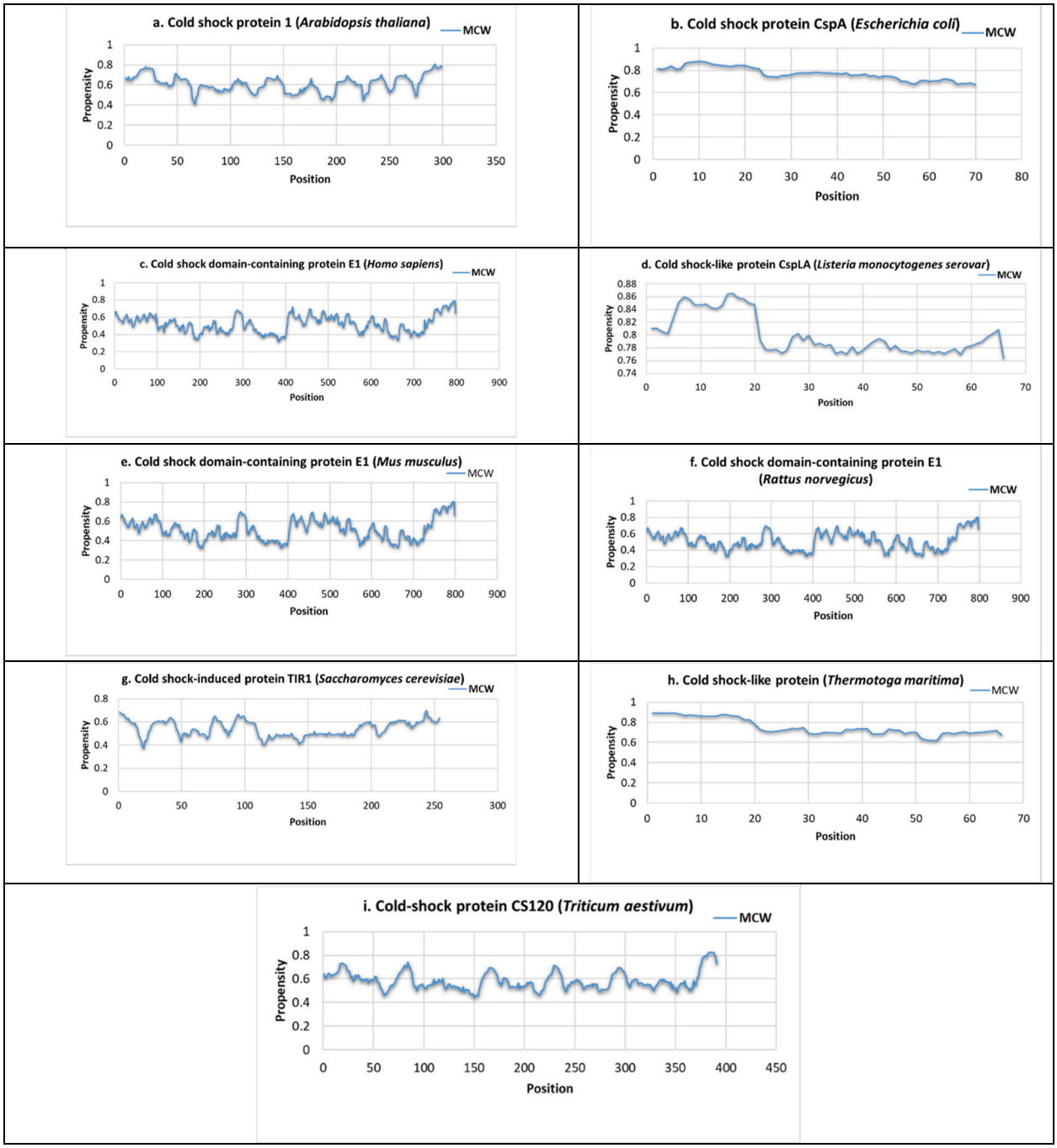

Fig. 5. Prediction of molecular recognition features (MoRFs). Propensity score of the proteins was predicted by MoRFCHiBi_Web (https://morf.msl.ubc.ca/index.xhtml) (Cut-off value 0.725) (a) Cold shock protein 1 (*Arabidopsis thaliana*), (b) Cold shock protein CspA (*Escherichia coli*), (c) Cold shock domain-containing protein E1 (*Homo sapiens*), (d) Cold shock-like protein CspLA (*Listeria monocytogenes serovar*), (e) Cold shock domain-containing protein E1 (*Mus musculus*), (f) Cold shock domain-containing protein E1(*Rattus norvegicus*), (g) Cold shock-induced protein TIR1 (*Saccharomyces cerevisiae*), (h) Cold shock-like protein (*Thermotoga maritima*), (i) Cold-shock protein CS120 (*Triticum aestivum*). Propensity scores shown in blue color. (For interpretation of the references to color in this ffgure legend, the reader is referred to the web version of this article.)

shown to undergo disorder-to-order transitions and bind to globular protein domains are called Molecular Recognition Features (MoRFs) [107–109]. Depending on folding types (α-helices, β-strands or stable coils), MoRfs have been categorized as α-MoRFs, β-MoRFs or σ-MoRFs. MoRFs play vital role in cell signaling and regulatory processes. Knowledge about their structure is useful in exploring biological functions alongwith disorder-based drug designing [110,111]. Identification of MoRFs is still a great challenge in computational biology. Several bioinformatics tools such as DISOPRED3 [112], MoRFpred [113], fMoRFpred [111], MFSPSSMpred [114], ANCHOR [115], $MoRF_{CHiBi\_Web}$ and $MoRF_{CHiBi}$ [116] have been developed with different prediction algorithms.

Molecular recognition features (MoRFs) locations in different cold shock protein sequences were predicted by $MoRF_{CHiBi\_Web}$ server. Propensity scores with residues index (Amino acid position) of different cold shock proteins are shown in Fig. 5.

Structure based drug designing relies on the known three-dimensional structure of the receptor, but disordered proteins lack a fixed structure. So, for structure-based drug screening against IDPs and IDRs novel strategies have to be applied as these proteins play key roles in various diseases. For active sites and drug target sites present in ordered regions of the IDPs structure based drug designing may be applied. But, for designing ligand against sites present in disordered regions one strategy may be to identify ligands that mimic the structure of the natural binding partner of these proteins. As, after binding with natural partners disordered regions adapt a fixed structure. The successful design of inhibitors against p53–Mdm2 protein is an interesting example of a disorder-based rational drug design approach [117]. Several that target IDRs for drug designing studies have been done and some are still ongoing [118–120]. This strategy may be applied for targeting IDRs present in the CSP family of proteins and for regulating the switches of key pathways in different diseases.

1.9. Evolutionary constraint of intrinsic disorders and CSPs

A study, performed to identify proteome-wide signatures of function in highly diverged intrinsically disordered regions, revealed that highly diverged disordered regions contain molecular features that are under evolutionary constraint. This could be important for function in IDR containing proteins like CSPs [121]. An attempt to identify sequence conservation in IDRs concluded that functionally constraint residues in protein-binding segments of the IDRs are relatively conserved as small signature motifs [122]. Another study reports that in IDRS the regions that interact with different partners in a protein interaction network, are more conserved. It was reported that at least 5 % of amino acids in disordered regions are important for the function [123]. An alignment of animal Y box proteins revealed that even N- and C-terminal domains are relatively conserved in chordates [31]. The multiple alignment of related CSPs suggested that the flanking domains are relatively less conserved regions in comparison to CSD. Despite being least conserved the constraint for conservation is higher in closely related organisms for example in Human YB1. Additionally, it seems that IDRs are relatively less conserved regions in distantly related homologs [31]. In the C-terminal region of human YB-1 alternatively arranged positively and negatively charged residues may have role in nonspecific binding DNA/RNA and with other proteins. Patterns rather than conserved amino acid sequences are prevalent in IDRs of CSPs [124]. An analysis of mammal Lin28 proteins reported that N-terminal cold-shock domain (CSD) and a zinc finger domain in the C-terminal are highly conserved. However, apart from zinc finger stretch the C-terminal region is rich in intrinsically disordered regions [125]. A comprehensive analysis of Human Y box proteins revealed that they consist an acidic Ala/Pro-rich N-terminal segment of variable length, a central highly conserved 68 AA (amino acid) CSD, and a variable C-terminal domain. The C-terminal has modules rich in arginine i.e. arginine-rich motifs (ARMs) and aromatic amino acids alternate with modules that are rich in acidic amino acids i.e. acid motifs (AcidMs), respectively. The N-terminal and C-terminal domains are intrinsically disordered and the CSD equilibrates between the native cold shock fold and the unfolded state particularly [126].

2. Conclusion

Cold shock proteins are highly conserved and diverse proteins in terms of structure and function, respectively. Generally, bacterial CSPs have fixed globular structure but their eukaryotic counterparts have additional flanking domains. Eukaryotic CSPs are pleiotropic in nature. Their CSD is generally involved in RNA/DNA binding but the flanking regions seem to play key role in binding with other biomolecules. This binding with a variety of interactors is crucial for pleiotropic role of the proteins. Analytics revealed that eukaryotic CSPs have intrinsically disordered regions prevalently localized in N- and C-terminal domains. So far, multifunctional role of CSPs has not been correlated with the abundance of IDRs within the proteins. Analytics also suggest that despite having variable regions some patterns, which may be based on type of amino acids or their order of arrangement, are present in closely related homologs of CSP IDRs. This article provides first-hand information for correlating pleiotropic role of CSPs with their intrinsically disordered regions. Since, human CSPs are known to play key role in diseases like Cancer and Alzheimer along with several other diseases, this categorization will provide a basis for adopting novel strategies to design therapeutics that have target sites present in IDRs of cold shock domain proteins.

CRediT authorship contribution statement

All the authors contributed in literature survey, analyzing data and writing the manuscript.

Declaration of competing interest

The authors report no conflicts of interest.

Data availability

No data was used for the research described in the article.

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