Coping with the cold: the cold shock response in the Gram-positive soil bacterium *Bacillus subtilis*

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All organisms examined to date, respond to a sudden change in environmental temperature with a specific cascade of adaptation reactions that, in some cases, have been identified and monitored at the molecular level. According to the type of temperature change, this response has been termed heat shock response (HSR) or cold shock response (CSR). During the HSR, a specialized sigma factor has been shown to play a central regulatory role in controlling expression of genes predominantly required to cope with heat-induced alteration of protein conformation. In contrast, after cold shock, nucleic acid structure and proteins interacting with the biological information molecules DNA and RNA appear to play a major cellular role. Currently, no cold-specific sigma factor has been identified. Therefore, unlike the HSR, the CSR appears to be organized as a complex stimulon rather than resembling a regulon. This review has been designed to draw a refined picture of our current understanding of the CSR in *Bacillus subtilis*. Important processes such as temperature sensing, membrane adaptation, modification of the translation apparatus, as well as nucleoid reorganization and some metabolic aspects, are discussed in brief. Special emphasis is placed on recent findings concerning the nucleic acid binding cold shock proteins, which play a fundamental role, not only during cold shock adaptation but also under optimal growth conditions.

Keywords: *Bacillus subtilis*; cold shock proteins; membrane; metabolism; nucleoid; ribosome

1. INTRODUCTION

Over 80% of the earth's biosphere is governed by permanent cold, i.e. temperatures below 5 °C (Russell 1990). Therefore, it is not surprising that many organisms of different species have evolved sophisticated mechanisms to overcome the life-endangering influence of low temperatures (Fujita 1999; Guy 1999; Hébraud & Potier 1999; Los & Murata 1999; Yamanaka 1999; Cavicchioli *et al.* 2000). In recent years, *Bacillus subtilis* has become a model organism for studies of the bacterial CSR representing the Gram-positive branch of mesophilic soil bacteria (Graumann & Marahiel 1999a). This extraordinarily versatile, rod-shaped micro-organism is genetically easily accessible and has been accepted as one of the best-studied bacteria even before its genome was entirely sequenced a few years ago (Kunst *et al.* 1997). *B. subtilis* is broadly distributed all over the world and lives in the upper layers of soil, where it is subject to every imaginable stress situation over the entire year. Besides its ability to form extremely resistant endospores in response to nutrient deprivation or slow dehydration (Stragier & Losick 1996), the facultative aerobe *B. subtilis* is capable of converting to anaerobic nitrate respiration under oxygen-limiting conditions (Hoffmann *et al.* 1995). By secreting highly active peptide antibiotics, it possesses a powerful self-defence system (Marahiel *et al.* 1993) and maintains active cell-to-cell communication during entry into the station-

ary phase, by exporting signal molecules to coordinate cellular growth with respect to reduced nourishment (Kaiser & Losick 1993). *B. subtilis*, possessing a growth optimum temperature of 39 °C and the capacity to grow within an overall temperature interval of 11–53 °C (Nichols *et al.* 1995), is excellently suited for analysis of the CSR, which is defined as the sum of all cellular reactions required for efficient adaptation to a sudden decrease in environmental temperature. In both *Escherichia coli* and *B. subtilis*, this process is characterized by a specific alteration of protein synthesis (Jones *et al.* 1987; Lottering & Streips 1995; Graumann *et al.* 1996). Interestingly, as reflected by transient growth arrest described for *E. coli* (Jones *et al.* 1987) and psychrotolerant *Yersinia enterocolitica* (Neuhaus *et al.* 2000), even above temperatures resulting in intra- or extracellular freezing, rapid temperature drops cause a variety of processes that affect the proper function of the membrane (transport, replication and energy production), protein folding, protein biosynthesis, primary metabolism and processes involving the biological information molecules RNA and DNA (Friedman *et al.* 1971; Broeze *et al.* 1978; de Mendoza & Cronan 1983; Jones *et al.* 1987; Graumann *et al.* 1996; Farewell & Neidhardt 1998). Consequently, for analysis of the CSR, the difference, ΔT , between the initial growth temperature and the temperature reached after cold shock is of importance rather than its final value post-shock (Jones *et al.* 1992a). The more dramatic this ΔT difference is, the more profound the observable effects that can be expected as far as changes in enzymatic activities, as well as membrane phase transitions, are concerned together with the demand for adaptational power. To account for this fact, in recent years, experimental cold shock conditions have been stan-

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standardized for mesophilic bacteria to base on an abrupt temperature downshift from 37 to 15 °C.

How do cells detect a decrease in temperature rapidly enough to activate an appropriate adaptation cascade to effectively prevent cease of growth and finally cellular death? To answer this question, it is advantageous to systematically separate the individual cellular processes of (i) detection of the incoming ΔT stimulus; followed by (ii) an appropriate regulatory signalling cascade; that results in (iii) a functional effective adaptation of protein synthesis; and finally enables the cells of some organisms (e.g. plants) to (iv) develop some kind of memory, which permits a much more rapid and efficient protection for these pre-adapted cells during freeze periods in the future. Briefly, what we are dealing with is a sequential arrangement of (i) one or more stimulus input device(s), (ii) a signal network, (iii) an output device, and (iv) a memory system.

We have summarized the most important aspects of this complex adaptational response in *B. subtilis*. After a short description of general mechanisms for temperature sensing and their relevance for this organism, we focus on the four major aspects (membrane, translation apparatus, chromosome adjustment and metabolism), with special attention devoted to CSPs. Finally, we outline some of the future perspectives in this research area.

2. TEMPERATURE PERCEPTION AND SIGNAL TRANSDUCTION

Cellular architectures offer three basic target structures, which possess thermodynamically sensitive properties that respond physically, almost immediately, to a rapid change in temperature, without interference of any protein or other factor(s). These are (i) phase transitions of membrane-bound lipids, (ii) conformational changes in proteins, and/or (iii) conformational changes in nucleic acids. These structures therefore represent excellent candidates for incorporation into rapid-responding temperature detection systems. In fact, for each of these structures, examples for specific functions in temperature sensing have been identified and described (Altuvia *et al.* 1989; Nagai *et al.* 1991; Hurme *et al.* 1997; Suzuki *et al.* 2000).

(a) *Membrane-bound two-component sensor transduction systems*

Because the cellular envelope comes into initial contact with a decrease in temperature, it appears reasonable to expect primary sensors of cold to reside in the membrane. Only recently, one of the 35 two-component signal transduction systems present in *B. subtilis* (Fabret *et al.* 1999) has indeed been identified as being involved in molecular thermosensing (Aguilar *et al.* 2001). This two-component system is encoded by the *desKR* operon (formerly *yocFG*; Kunst *et al.* 1997), which is located directly downstream of the cold-inducible FA desaturase gene *des* (formerly *yocE*; Kunst *et al.* 1997) and was shown to be non-essential under standard conditions (Fabret & Hoch 1998). The predicted protein products of *desK* and *desR* exhibit a sequence similarity to membrane-bound histidine kinases containing four transmembrane segments (*E. coli* NarL type) and cytosolic response regulators, respectively (Fabret *et al.* 1999). *desKR* has been demonstrated to be responsible for

transient cold-induced expression of *des*, regulated at the transcriptional level via specific recognition of a dyad-symmetry-containing sequence element located upstream of the *des* -10 promoter region, by the action of response regulator DesR (Aguilar *et al.* 2001). In accordance with properties of other sensor kinases (Dutta *et al.* 1999), DesK is proposed to possess dual functionality acting either as kinase or as phosphatase to phosphorylate or dephosphorylate DesR in a temperature-dependent manner, respectively (Aguilar *et al.* 2001). Based on the observation that, currently, no response regulator has been found to be active in its unphosphorylated form (Hoch 2000), the current model assumes that at 37 °C DesK acts as a phosphatase to keep DesR in an inactive dephosphorylated form. After cold shock, DesK activates DesR by phosphorylation to recognize and bind to the *des* promoter region and initiate transcription, which reaches a maximum at *ca.* 30 min post-shock (Aguilar *et al.* 1999). Production of Des finally results in synthesis of lipid-incorporated UFAs, which, in turn, shut down *des* expression by converting DesK back to its phosphatase state rather than directly interacting with DesR (Aguilar *et al.* 2001). It seems possible that an UFA-mediated increase of membrane fluidity results in a conformational change in membrane-bound DesK thereby triggering the kinase to phosphatase conversion.

Presently, one of the most exciting questions is whether the DesKR thermosensor represents the universal key-temperature perception system in *B. subtilis*, or whether it is specific for cold-induced expression of *des*. However, even in the presence of a highly functional and rapidly responding membrane-bound signal transduction system, the temperature insulating properties of a typical phospholipid bilayer can be expected to be insufficient to prevent quick cytosolic progression of temperature reduction. Therefore, cells that harbour alternative temperature-sensing capabilities, would have a considerable advantage for survival.

(b) *Translation apparatus and stringent factor RelA*

In fact, at least two lines of evidence support the idea that other membrane-dependent and/or membrane-independent temperature detection systems may exist in *B. subtilis*, which appear to work in parallel to DesKR. First, under the conditions examined, disruption of the *desKR* operon showed no detectable phenotype after cold shock indicating dispensability of this thermosensor (Aguilar *et al.* 2001). Second, as demonstrated for *E. coli* (VanBogelen & Neidhardt 1990), a cold shock-like response can be induced at 37 °C in *B. subtilis* by the addition of sub-lethal amounts of translation inhibitor chloramphenicol (Graumann *et al.* 1997) suggesting that blocking of the ribosome triggers, at least in part, the CSR. This observation indicates that the translation apparatus itself might participate as a second temperature perception system. However, it remains unclear whether intrinsic properties of the ribosome, or mRNAs, or both, simply allow for a preferential translation of specific mRNAs encoding cold-induced proteins or whether, in addition to this, a currently undetected ribosomal regulatory signal is generated.

Interestingly, after cold shock treatment, increased syn-

thesis of (p)ppGpp has been reported for *B. subtilis*, albeit under rather unphysiological conditions (Ikehara *et al.* 1984). This is in contrast to the situation reported for *E. coli*, where (p)ppGpp levels decrease after cold shock (Pao & Dyess 1981; Mackow & Chang 1983) but might reflect other RelA/Spot-related differences between Gram-positive and Gram-negative organisms (Wendrich *et al.* 2000). It has recently been demonstrated, that in *B. subtilis* this signal appears to be generated solely by stringent factor RelA (Wendrich & Marahiel 1997). In *E. coli* RelA resides at the ribosome (Haseltine *et al.* 1972) and, possibly in concert with ribosomal protein L11, is believed to scan the ribosome for uncharged tRNAs bound to its cognate mRNA as a result of amino acid starvation. After detection of uncharged tRNA, RelA rescues ribosome blockage by removing the tRNA and simultaneously synthesizing the (p)ppGpp alarmone (Haseltine *et al.* 1972). This effector then binds to key enzymes, such as RNA polymerase, to modulate protein function and alter gene expression (Cashel *et al.* 1996). Comparison of high-resolution protein expression patterns of cold-shocked (15 °C) and amino acid starved *B. subtilis* (37 °C) clearly shows that the overall expression profiles differ significantly. However, it should be noted that some of the proteins induced in *B. subtilis* after cold shock (e.g. CysK and IlvC which are involved in amino acid biosynthesis; Graumann *et al.* 1996), are also induced during stringent response of the wild-type but not in a *relA* deletion mutant (Wendrich & Marahiel 1997). These observations clearly indicate a regulatory role of (p)ppGpp level for expression of the corresponding genes. Whether alteration of (p)ppGpp levels in either direction after cold shock is affecting the CSR in *B. subtilis* has yet to be explored. However, the results obtained for *E. coli* are encouraging, since for this organism a clear impact of (p)ppGpp level on gene expression after cold shock has been established (Jones *et al.* 1992a).

(c) DNA topology

A third system, capable of serving as a temperature sensor and thereby directly affecting gene expression, is the DNA molecule itself. Although the Lk in an isolated closed circular DNA molecule is a topological parameter that cannot be changed, upon heating DNA tends to denature which results in decreasing local Tw of both strands around each other. The corresponding unwinding angle has been measured to be $-0.01 \text{ deg } ^\circ\text{C}^{-1}$ per base pair (Charbonnier *et al.* 1992; Duguet 1993). For a covalently closed DNA molecule having constant Lk, compensatory coiling of the helix in space, which is termed Wr, is generated ($\Delta\text{Lk} = \Delta\text{Tw} + \Delta\text{Wr}$). Consequently, after cold shock DNA would physically tend to overtwist and, correspondingly, create negative supercoiling ($\Delta\text{Wr} < 0$). Such an overtwist is locally interpreted as Lk excess, and will attract topoisomerases decreasing Lk such as gyrase that will then establish global negative supercoiling. This is in line with the experimental fact that, in *B. subtilis*, an increase in the negative supercoiling of DNA has been observed after cold shock without significant alteration of gyrase subunit *gyrA* and *gyrB* mRNA levels (Krispin & Allmansberger 1995), even under conditions that block protein *de novo* synthesis (Aguilar *et al.* 1999). Since it is well known that DNA topology significantly

modulates the location of -35 and -10 promoter regions relative to each other this mechanism provides a powerful means of gene expression after cold shock for all organisms. Interestingly, the gyrase subunit GyrA is one of the cold-induced proteins in *E. coli* (Jones *et al.* 1992b). However, such cold induction is not generally required for the introduction of a global negative DNA supercoil since it has been shown that topoisomerases are temperature-optimized enzymes with gyrase having a higher activity at lower temperatures.

In summary, the cold shock stimulon in *B. subtilis* seems to be controlled by at least three different types of temperature perception systems that reside in the membrane, the ribosome and the DNA (figure 1). However, the precise molecular organization of this complex signalling network requires much further investigation.

3. MEMBRANE ADAPTATION

For all organisms, maintenance of functional cellular membranes is a limiting factor for survival. Numerous investigations in the past have shown that biomembranes are highly complex structures that exist in several physically different states (Singer & Nicolson 1972; Israelachvili *et al.* 1980; Cevc 1991; Dowhan 1997). After cold shock, membrane fluidity is rapidly reduced which, depending on the lipid composition, results in local, and later global, phase transition from a liquid-crystalline state to a more rigid gel-like state. Such phase transitions affect membrane-coupled processes mediated by lipid-embedded proteins. To restore membrane functionality, with decreasing temperature, FAs of lower melting points are incorporated into lipids in a species-specific mode. In principle, there are three mechanisms to achieve modification of the membrane fluidity, generally termed homeoviscous adaptation (Sinensky 1974). These include FA acyl chain-length alteration, FA acyl chain branching and the introduction of usually *cis*-configured carbon double bonds (de Mendoza & Cronan 1983; Russell 1984; de Mendoza *et al.* 1993). Currently, shortening and methyl branching of FAs have been reported to be strictly dependent on FA *de novo* biosynthesis (Suutari & Laakso 1994). The introduction of double bonds into acyl chains, however, can either be achieved anaerobically, during FA synthesis as demonstrated for *E. coli* (Cronan & Rock 1996) or aerobically, by modification of existing FAs through FA desaturase enzymes, as shown for bacilli (de Mendoza *et al.* 1993; Los & Murata 1998). In contrast to *E. coli* and Gram-negative bacteria in general, lipids of Gram-positive bacilli contain a high proportion of iso- and anteiso-branched FA species. These molecules are derived from valine, leucine and isoleucine precursors resulting in iso-branched-even-chain, iso-branched-odd-chain and anteiso-branched-odd-chain FA species (Bishop *et al.* 1967; Kaneda 1991). This fact reflects a tight coupling of amino-acid metabolism and FA biosynthesis.

After cold shock, *B. subtilis* has been shown to utilize a dual strategy to restore membrane fluidity. In parallel with the initiation of massive *de novo* synthesis of saturated anteiso-branched C-15 and C-17 FAs for long-term adaptation (Klein *et al.* 1999), *B. subtilis* transiently induces the unique FA desaturase gene *des* (Aguilar *et al.* 1998, 1999). The presence of this gene is essential for the syn-

Figure 1. Organization of the *B. subtilis* cold shock stimulon. The membrane, translation apparatus and DNA may serve as sensors whose physical properties (membrane lipid-phase transition, conformational alteration of the ribosome, local change in DNA topology) are altered upon a reduction in the environmental temperature which generates a signal (phosphorylation of a two-component system response regulator, alteration of (p)ppGpp level and activation of promoter) that finally allows for an adaptational response at the protein level.

thesis of all four unsaturated FA species so far detected in this bacterium (Weber *et al.* 2001a). Although earlier investigations assigned *B. subtilis* desaturase activity to a membrane-associated enzyme of the acetyl-CoA-desaturase type (Dunkley *et al.* 1991), it has now become clear that this enzyme is a lipid desaturase (Grau & de Mendoza 1993). As such, it modifies the lipids present in the membrane directly at the time of cold shock, thereby providing a rapid-responding system for the modulation of membrane fluidity. Interestingly, in the absence of exogenous isoleucine sources, deletion of *des* in a *B. subtilis* strain JH642 background results in a cold-sensitive growth phenotype, indicating an important role for this enzyme during cold adaptation (Weber *et al.* 2001a). In such a mutant, artificial expression of *des in trans* does not only cure the observed phenotype but demonstrates that Des possesses cryoprotective properties that significantly increase the cold tolerance of *B. subtilis* (Weber *et al.* 2001a). Although it was reported that UFAs are exclusively synthesized upon cold shock (Aguilar *et al.* 1998, 1999), recent investigations revealed that in the absence of isoleucine, *B. subtilis* produces detectable amounts of UFAs even at 37 °C (Weber *et al.* 2001a). These findings indicate that our current understanding of the regulation of UFA production is not yet complete and might involve isoleucine itself as a modulator molecule.

4. CSPs AND TRANSLATION APPARATUS

(a) Structure and function of CSPs

The most strongly cold-induced proteins currently identified belong to the widespread family of small, nucleic acid binding CSPs and represent the prototype of the cold shock domain, which is conserved from bacteria to man (Wistow 1990; Wolffe *et al.* 1992). The cold shock domain harbours the nucleic acid binding motifs, RNP-1 and RNP-2 (Landsman 1992). Currently, CspB from *B.*

subtilis (Schindelin *et al.* 1993; Schnuchel *et al.* 1993), CspA from *E. coli* (Newkirk *et al.* 1994; Schindelin *et al.* 1994), CspB from thermophile *Bacillus caldolyticus* (Mueller *et al.* 2000) and TmCsp from hyperthermophile *Thermotoga maritima* (Kremer *et al.* 2001) have been structurally determined using crystallography and/or NMR techniques (figure 2). CSPs, with a length of ca. 70 amino acids, share a highly similar overall fold. This fold consists of five antiparallel β -sheets, forming a β -barrel structure, with surface exposed aromatic and basic residues (RNP-1 and RNP-2 motifs on β -strands 2 and 3, respectively). These residues have been shown to be responsible for the nucleic acid binding properties of this protein family whose members possess variable binding affinities and sequence selectivities (Graumann & Marahiel 1994; Schröder *et al.* 1995; Hanna & Liu 1998; Lopez *et al.* 1999; Phadtare & Inouye 1999; Lopez & Makhatadze 2000). With the general exception of archaea and cyanobacteria, this protein family has been identified in almost all psychrotrophic, mesophilic, thermophilic and hyperthermophilic bacteria so far examined, and is also present in the earliest diverging bacterial branches *Thermotoga* and *Aquifex* which indicates an ancient origin (Graumann & Marahiel 1998). CSPs are often found in differentially regulated multiple copies of varying homologies within a given bacterial species (Yamanaka *et al.* 1998) and some of them have been reported to form dimers *in vitro* (Schindelin *et al.* 1993; Makhatadze & Marahiel 1994; Mayr *et al.* 1996; Yamanaka *et al.* 2001; figure 2).

In *B. subtilis*, three cold-inducible CSPs have been identified (CspB-D) while in *E. coli* only four (CspA, CspB, CspG and CspI; Wang *et al.* 1999), out of a total of nine CSPs (CspA-I), are cold induced (Yamanaka 1999). In spite of a very similar overall fold, the reported individual differences in CSP : CSP and CSP : RNA/DNA interactions may also reflect different func-

Figure 2. Stereo structure of CspB from *B. subtilis* (PDB accession no. 1CSP; Schindelin *et al.* 1993). (a,b) Ribbon and (c,d) surface charge models of monomeric and dimeric CspB, respectively. Models were generated with Swiss-PdbViewer v. 3.7b2 (Guex & Peitsch 1997) and ribbon diagrams were rendered using POV-Ray v. 3.1g. Assuming fully charged residues, the ionic strength of the solvent was set to zero, and surface charges were calculated according to the Poisson-Boltzmann potential with -1.8 kT e^{-1} or less (shown in red), neutral potential (shown in white) and $+1.8 \text{ kT e}^{-1}$ or more (shown in blue). In the ribbon diagrams, side-chains of residues Lys7, Trp8, Lys13, Phe15, Phe17, Phe 27, His29, Phe30 and Arg 56 are indicated, which were examined by mutation analysis with respect to the contribution to nucleic acid binding (Schröder *et al.* 1995). Interestingly, the shape of the dimer appears as a protein mimic of one strand of a DNA duplex with the presumed nucleic acid binding sites presented in a twinned position relative to each other and a broadly stretched, negatively charged backbone.

protein	amino acid sequence	amino acid number	pI/MW (kDa)
	<div> <div>β-1</div> <div>β-2</div> <div>β-3</div> <div>β-4</div> <div>β-5</div> </div>		
CspB_Bs	ML---EKKVKMFNENKGGGIEVEGQ-DDVFVHFSAIQGGFKTLEGGQAVSFEIIVGNGRGPQAAVNTKEA-----	67	4.54/7.37
CspD_Bs	MQ---NKKVKMFNENKGGGIEVEGQ-DDVFVHFSAIQGGFKTLEGGQAVSFEIIVGNGRGPQASNVVKL-----	66	4.51/7.31
CspC_Bs	MB---QETVKMFNENKGGGIERENG-DDVFVHFSAIQSDGFKSLDEGQKVSFEDVEGQARGQAQAAVQKA-----	66	4.72/7.25
CspD_Ec	ME---KETVKMFNNAKGGGIEGEGEDITAAVYSTIQMDGYRTIKAGQSVQFEDVHQPKNHNASVIVPVEVEAAVA	74	5.81/7.97
CspA_Ec	MSGKMTGLVKMFNADKGGGIEITDDGSKDVFVHFSAIQNDGYKSLDEGQKVSFEITGSCAKGPAAAGNVTSL-----	70	5.58/7.40
CspB_Ec	MSNKMTGLVKMFNADKGGGIEITDDGSKDVFVHFSAIQNDNYRTLFEGQKVTESITGSCAKGPAAAGNVITD-----	71	6.54/7.72
CspG_Ec	MSNKMTGLVKMFNADKGGGIEITDDGSKDVFVHFSAIQSNFRFTINENKQVEFSEIQQGQGPAAAGNVVTL-----	70	5.64/7.78
CspI_Ec	MSNKMTGLVKMFNENKGGGIEITDDGSKDVFVHFSAIQSNDFKTLITENDEVEFGIENGPKGPAAVHVVAL-----	70	6.03/7.68
CspC_Ec	MA-KIKGVKMFNENKGGGIEITADGSKDVFVHFSAIQNGGFKTLAEGQNVSEFETQDQKGPAAAGNVTAI-----	69	6.54/7.40
CspE_Ec	MS-KIKGVKMFNENKGGGIEITEDGSKDVFVHFSAIQTNQFKTLAEGQNVSEFETITGAKGPAAAGNVIAL-----	69	8.09/7.46
CspF_Ec	MSRKMTGLVKTFDGKSEKGLITSDGRIDVQLVHSALNLRDAEEITTLRVSECRINLGRGPAAAGNVYLS-----	70	9.51/7.56
CspH_Ec	MSRKMTGLVKTFDRKSEKGLITSDGRKEVQLVHSALNLRDAEEITTLRVSECRVNLGRGPAAAGNVYLS-----	70	10.28/7.72
majority	MS-KMTGLVKWFNAEKGGGIFITPDGSKDVFVHFSAIQGGFKTLEGGQKVEFEIENGAKGPAAAGNVVAL-----		

Figure 3. Classification of the CSP family from *B. subtilis* and *E. coli*. Alignment of CSP family members from *B. subtilis* (CspB-D_Bs) and *E. coli* (CspA-I_Ec) using the CLUSTAL W algorithm (Thompson *et al.* 1994) allows for a clear differentiation of five subgroups. Individual MW and isoelectric point (pI) of each CSP as well as the location of the five antiparallel β -strands (as determined for CspB from *B. subtilis*) and RNP-1 and RNP-2 motifs are indicated. Residues identical to 100%, at least 75% or at least 50% in all CSP sequences are highlighted in black, dark grey and light grey, respectively.

tions *in vivo*. As shown in figures 3 and 4, alignment of the two CSP families from *E. coli* and *B. subtilis* followed by construction of a phylogenetic tree, allow for a clear definition of five distinct CSP subgroups. Interestingly, these groups cluster according to *csp* origin and expression pattern, and comprise of the highly homologous *B. subtilis* CSPs CspB-CspD, the cold-inducible CSPs CspA, CspB, CspG and CspI (Wang *et al.* 1999), constitutively expressed CspC and CspE (Yamanaka *et al.* 1994), stationary phase and nutritional starvation induced CspD (Yamanaka & Inouye 1997), and the currently uncharac-

terized, highly basic CSPs CspF and CspH from *E. coli*. This assignment of different CSPs to distinct subgroups is also reflected in their different functions, as currently determined. Apart from its properties as a transcriptional activator (Brandi *et al.* 1994), CspA from *E. coli* has also been shown to act as an RNA chaperone *in vitro* (Jiang *et al.* 1997) and possesses transcriptional antiterminator functions like its homologues CspC and CspE (Bae *et al.* 2000). Moreover, CspC and CspE were found to be implicated in promoting or protecting chromosome folding and to act as high-copy suppressors of mutations in

Figure 4. Classification of the CSP family from *B. subtilis* and *E. coli*. Phylogenetic tree of CSP family members from *B. subtilis* (Bs) and *E. coli* (Ec) as generated without further distance corrections from the alignment displayed in figure 3 using TREEVIEW v. 1.65 (Page 1996). Gram-positive CSPs are very homologous whereas among Gram-negative CSPs a significant structural diversity exists that is also reflected by diverging cellular functions, as yet examined. Scale bar, 0.1.

the chromosomal-partition gene *mukB* (Yamanaka *et al.* 1994; Hu *et al.* 1996). CspD, which appears to exist exclusively as a homodimer, is specifically expressed in the stationary phase and has been shown to inhibit replication (Yamanaka & Inouye 1997; Yamanaka *et al.* 2001).

The construction and analysis of combined *csp* deletion mutants in *B. subtilis* have demonstrated that the presence of at least one copy of this protein family is essential, under optimal growth conditions as well as during cold shock (Graumann *et al.* 1997). It is also evident that a hierarchy of importance exists, in which CspB appears to be most important, followed by either CspD or CspC depending on optimal or low-temperature growth conditions, respectively. An analysis of a *cspB/cspC* double-deletion mutant revealed that removal of these *csp* genes results in pleiotropic alteration of protein synthesis, cell lysis during entry into the stationary growth phase, alteration of nucleoid structure, and the inability to differentiate to endospores indicating a fundamental yet poorly understood function for CSPs (Graumann *et al.* 1997; Weber *et al.* 2001c). Cell lysis upon entry into the stationary phase might be explained by the observation that both CspB and CspC are stationary-phase induced which, in the double mutant, is no longer possible and shows that in *B. subtilis*, CSPs are not only inducible after cold shock (Graumann & Marahiel 1999b). Interestingly, among the processes which cold shock and stationary phase have in common, at least in *E. coli*, is rapid inactivation of ribosomal function (Jones & Inouye 1996; Wada 1988) which, in both *E. coli* and *B. subtilis*, is discussed as one of the possible signals triggering CSP induction (VanBogelen & Neidhardt 1990; Graumann *et al.* 1997; Etchegaray &

Inouye 1999). Only recently, on the basis of their highly similar fold (Sette *et al.* 1997), heterologous expression of translation initiation factor IF1 from *E. coli* in a *B. subtilis* *cspB/cspC* double-deletion strain was shown to cure both the growth and sporulation defects observed for this mutant. This suggests that IF1 and CSPs have, at least in part, overlapping cellular function(s) (Weber *et al.* 2001a). Because the initiation step in bacterial protein biosynthesis requires the translation initiation factor IF1, which binds directly to the 30S subunit of the ribosome, as demonstrated recently by co-crystallization experiments (Gualerzi *et al.* 2000 and references therein; Carter *et al.* 2001), these data might suggest a first potential connection between CSPs and the initiation of translation. This process is believed to represent the bottleneck of bacterial adaptation during cold shock (Friedman *et al.* 1971; Broeze *et al.* 1978; Jones & Inouye 1996; Farewell & Neidhardt 1998). Interestingly, for *B. subtilis* as well as for *Bacillus stearothermophilus*, expression of an IF1 protein homologue has not yet been reported (Kay *et al.* 1976). It is therefore tempting to speculate as to whether CSPs in Gram-positive bacteria might act as general, or at least alternative, translation initiation factors under certain stress conditions. In fact, *in vivo* localization studies using immunofluorescence techniques and a fusion of green fluorescent protein to the C-terminus of CspB revealed that CSPs co-localize with ribosomes in *B. subtilis* (Mascarenhas *et al.* 2001; Weber *et al.* 2001c). This is in line with the observations reported for *Streptomyces aureofaciens* in which CSPs were co-purified with ribosomes (Mikulik *et al.* 1999). However, since resolution of fluorescence microscopy is rather low, and the reported copurification results might also be explained by interaction of CSPs with ribosome-bound mRNA, rather than by a direct CSP : ribosome interaction, at this stage alternative explanations should be considered.

In an early work, apart from its evident binding to the ribosomal decoding site during the translation initiation process, IF1 was shown to alter the structure of various oligonucleotides in terms of disrupting nucleic acid interactions *in vitro* (Schleich *et al.* 1980). These data may be interpreted as a putative chaperone activity, as shown *in vitro* for CspA from *E. coli* (Jiang *et al.* 1997) and as suggested for *B. subtilis* CSPs (Graumann *et al.* 1997). Furthermore, cross-linking studies have shown that IF1 binds to mRNA *in vivo*, although it is unclear whether this interaction occurs during the translation initiation step or reflects the activities of non-ribosomal-associated IF1 comparable to RNA chaperones (Schouten 1985a,b). In conclusion, the data presented above can be interpreted in accordance with a previously proposed RNA chaperone model for CSP function (Graumann *et al.* 1997), or alternatively, give rise to a novel hypothesis of CSPs acting as translation initiation-like factors in *B. subtilis* and, possibly, also other bacteria (Weber *et al.* 2001b). Based on the fact that, after cold shock only CSPs are produced under conditions that totally block protein synthesis (100 µg ml⁻¹ of kanamycin or 200 µg ml⁻¹ of chloramphenicol; Etchegaray & Inouye 1999), in this context, a novel two-step model could be imagined. Cold-inactivated ribosomes first exclusively synthesize CSPs (e.g. due to intrinsic properties of their mRNAs), which then alter ribosome conformation thereby enabling synthesis of CIPs

such as CsdA and RbfA which finally allow for resumption of synthesis of bulk protein. However, all these results make a function of Gram-positive CSPs in coupling of transcription to translation probable, which is in remarkable analogy to the evidence reported for eukaryotic Y-box proteins, which have an N-terminal CSD in common with bacterial CSPs (Wolffe *et al.* 1992; Sommerville 1999).

(b) *Modulation of translation apparatus*

One of the striking differences between *E. coli* and *B. subtilis* during cold shock adaptation is the fact that *E. coli* transiently stops growing after cold shock, while *B. subtilis* continues to grow, albeit at a significantly reduced doubling rate. During this growth arrest, which is termed the cold-acclimatization phase, *E. coli* induces a set of ribosome-associated proteins such as the DEAD-box RNA helicase CsdA (Jones *et al.* 1996) and the 30S ribosomal binding factor RbfA (Jones & Inouye 1996). In common with CSPs, these proteins are preferentially processed under conditions where mRNAs of bulk protein are barely accepted as ribosomal substrates, and are believed to modify the translational apparatus to allow for resumed synthesis of bulk proteins. CsdA, for example, was shown to destabilize double-stranded RNA secondary structures *in vitro* (Jones *et al.* 1996), which are more stable at lower temperatures and may pose a problem during recognition of mRNA translation initiation sites by ribosomes. Conversely, RbfA was originally identified as a multicopy suppressor of a cold-sensitive 16S rRNA mutation and binds to the 30S subunit (Dammel & Noller 1995), which might cause a readjustment of cold-induced conformational change of the ribosome, thereby positively affecting translation initiation.

Although the *B. subtilis* genome encodes homologues for both CsdA (YdbR) and RbfA (RbfA), synthesis of these proteins has not yet been resolved by 2D gel electrophoresis, possibly due to their basic pI. However, after cold shock *B. subtilis* induces or accumulates a variety of other ribosomal or ribosome-associated proteins such as S6, L7/L12, L10, EF-G, EF-Ts and EF-Tu (Graumann *et al.* 1996; Graumann & Marahiel 1999a). Since these components are part of the standard translation apparatus, and not cold-specific add-ons as reported for *E. coli*, it is tempting to speculate whether, upon cold shock, *B. subtilis* assembles a novel subfraction of ribosomes, possibly by incorporating different rRNAs, or simply increases the pool of ribosomes in the cell to facilitate translation efficiency. Only recently, it was shown that the 10 rDNA operons present in *B. subtilis* are differentially regulated in response, for example, to growth-phase changes (Kinoshita *et al.* 2001). Interestingly, a closer inspection of a 16S rRNA region which we term 'loop 190' revealed that it is complementary to a section including, in part, the highly conserved cold-box element that is found near the 5' terminus of the unusually long 5'UTRs of *B. subtilis* *cspB* and *cspC* mRNAs (Weber 1998). Comparison of 16S rDNA sequences from *B. subtilis* to corresponding data known for the 16S rRNA structure from *E. coli* suggests that this region might represent, at least in part, single-stranded RNA and may therefore be available for base pairing. In *E. coli* 'loop 190' is located near ribosomal protein S20 (identical to ribosomal protein L26; Wittmann-

Liebold 1986), which resides at the interface between the small and large ribosomal subunit and remains attached to either the 30S or the 50S subunit when the ribosome is dissociated. In the latter case, regions of 16S rRNA that were previously masked by protein S20 might now become even more accessible for a potential interaction with the 5'UTR of the *B. subtilis* *cspB* mRNA. Therefore, it would be interesting to test whether 'loop 190' can serve as an additional Shine-Dalgarno-like interaction site, which might generally explain preferential translation of *csp* mRNAs by cold-shocked ribosomes in bacteria. Although the details remain to be experimentally elucidated, in this context it is worth emphasizing that a specific signature in 16S rDNA was identified that overlaps with 'loop 190' and was shown to allow for discrimination between mesophilic and psychrotrophic *Bacillus* strains (Prüß *et al.* 1999).

5. NUCLEOID STRUCTURE AND TRANSCRIPTION

Although the size and shape of *B. subtilis* cells do not change detectably after cold shock, microscopic analyses have revealed that cold shock results in nucleoid compaction, as is also the case at 37 °C upon entry into the stationary growth phase (Weber *et al.* 2001c). Such compaction might be interpreted in terms of classical gyrase-mediated increase in negative DNA supercoil, increased amounts or altered activity of other DNA structuring proteins, and/or reduced synthesis of proteins that are directed towards the membrane that is thought to expand the nucleoid as a result of expansion forces mediated by coupling transcription and translation to membrane insertion (Woldringh *et al.* 1995).

(a) *Reorganization of nucleoid structure*

It is well known that negative supercoiling facilitates DNA-duplex opening thereby exposing chromosomal regions of ssDNA that might enhance transcription and replication in *B. subtilis*. Interestingly, the *E. coli* *cspA* gene reported to be cold inducible at the transcriptional level, as well as cold-inducible *cspB* and *cspC* genes in *B. subtilis*, contain an AT-rich UP-element (Fang *et al.* 1997; Goldenberg *et al.* 1997; Graumann *et al.* 1997; Mitta *et al.* 1997; Kaan *et al.* 1999) that, apart from being directly recognized by the α -subunit of RNA polymerase as demonstrated for *E. coli* (Ross *et al.* 1993), might serve as a strand-opening hot-spot during cold shock. In fact, in *B. subtilis*, the negative supercoil of the DNA increases after cold shock (Grau *et al.* 1994; Krispin & Allmansberger 1995). The addition of gyrase inhibitor novobiocin, however, caused the abolition of the cold-induced expression of desaturase gene *des*, which clearly indicates an important gene regulatory role for gyrase-mediated changes in DNA topology during cold shock adaptation in *B. subtilis* (Grau *et al.* 1994). This observation, together with the DesKR functionality described above, indicates that after cold shock, at least in the case of *des*, the status of the membrane is compared with the status of the nucleoid before a productive transcriptional activation of a cold-inducible target gene is initiated. Because of the intrinsic properties of mRNA, or cold-shocked ribosomes, or both, the resulting mRNA might then preferentially be selected for translation from a pre-existing mRNA pool to finally yield the cold-required protein.

Among other factors known to alter nucleoid structure in *B. subtilis* are histone-like protein HBSu (Köhler & Marahiel 1997), SMC (Graumann 2001), the family of SASPs (Griffith *et al.* 1994), and *E. coli* Dps homologues MrgA (Chen & Helmann 1995) and Dps (Antelmann *et al.* 1997). Interestingly, although in *B. subtilis* none of these proteins has been examined with respect to a function during the cold shock response, it was shown that in *E. coli* gyrase and HU proteins (HBSu homologues) function in concert (Mizushima *et al.* 1997). In fact, HU proteins are essential during cold shock adaptation of *E. coli* (Wada *et al.* 1988) and in *Mycobacterium smegmatis* a histone-like protein is one of the major cold-induced proteins (Shires & Steyn 2001). Since SspF is currently the only SASP to which no specific function has been assigned during sporulation (Loshon *et al.* 1997), it might be interesting to investigate whether it is of relevance to the cold shock response—especially in conjunction with HBSu which was shown to modulate the effects of SASPs, at least *in vitro* (Ross & Setlow 2000). These data suggest that even in *B. subtilis* DNA structuring proteins are likely to play a role after cold shock.

Although the model proposed by Woldringh *et al.* (1995) offers an additional explanation for a more condensed chromosome after cold shock, some experimental observations indicate that the structure of the nucleoid is influenced by the transcription process itself. This is evident since in contrast to the addition of translation inhibitor chloramphenicol, which results in nucleoid compaction, it was shown that for *B. subtilis* the addition of RNA polymerase inhibitor rifampicin results in expansion of the nucleoid (Weber *et al.* 2001c). According to Woldringh's model, the inhibition of transcription should lead to a more condensed nucleoid since under such conditions expansion forces mediated by translation of mRNAs encoding membrane-directed proteins are abolished.

Interestingly, in growing *B. subtilis* cells, the reported subcellular localization of CSPs and ribosomes to areas surrounding the nucleoid, is dependent on active transcription and consequently on nucleoid structure, since upon addition of rifampicin or entry of cells into the stationary growth phase, CSPs and ribosomes localize throughout the cell (Mascarenhas *et al.* 2001; Weber *et al.* 2001c). Conversely, a lack of CSPs in *csp* double mutants leads to nucleoid compaction (Weber *et al.* 2001c) that, according to Woldringh's model, might be a result of compromised protein synthesis in such strains (Graumann *et al.* 1997). Therefore, the function of CSPs, in terms of coupling transcription to translation, is somehow interconnected to nucleoid structure. This might provide a first clue as to why two CSPs in *E. coli* were identified as multi-copy suppressors of a mutation in condensation factor MukB (Yamanaka *et al.* 1994), and confer resistance against chromosome decondensation through camphor (Hu *et al.* 1996).

(b) *The problem of R-loop formation during transcription*

Apart from global negative DNA supercoiling as introduced by gyrase, local negative as well as local positive supercoiling is generated behind and ahead of the elongating RNA polymerase, respectively (Liu & Wang 1987). Although this is also a problem under optimal growth con-

ditions, low transcription velocities, as well as decoupling of transcription to translation, favour the annealing of nascent RNA with the DNA-template strand behind the moving RNA polymerase (Massé & Drolet 1999). After cold shock, both generally reduced reaction rates as well as hampered protein synthesis are evident. Although topoisomerase I and RNase H have been shown to be involved in resolving R-loop formation in *E. coli* (Massé & Drolet 1999), it would not be surprising if massively cold-induced proteins as small as CSPs play an additional role in preventing the formation of such loops.

6. METABOLISM, PROTEIN FOLDING AND CELL DIFFERENTIATION

(a) *Metabolism*

To maintain the complex enzymatic reaction network under cold shock conditions, where some enzymatic activities remain essentially intact while others possibly collapse, *B. subtilis* can be expected to adjust the synthesis of metabolically important proteins. Indeed, it was shown that several proteins involved in the phosphotransferase system, amino acid biosynthesis, glycolysis, lipid metabolism, nucleotide biosynthesis, pentose phosphate cycle and riboflavin synthesis are cold induced or accumulate upon temperature reduction (Graumann *et al.* 1996). In this context it is interesting to note that in cold-shocked *B. subtilis* cells, the ATP : ADP ratio as a simplified term for cellular energy load, as well as the GTP level, has not yet been examined. Both values might prove useful as an additional metabolic signal for gene regulation and modulation of protein activity as, for example, discussed for gyrase.

(b) *Protein folding in the cold*

Since peptidyl-prolyl bonds in proteins cannot rotate freely, but in active proteins need to adopt either *cis* or *trans* configuration, isomerization of these two states is a rate-limiting step in protein folding and requires enzymatic catalysis. Therefore, like topoisomerases and RNA chaperones that are required after cold shock to adjust, prevent or resolve excessive structuring of nucleic acids, it seems probable that protein chaperones are required in the cold. Indeed, in *B. subtilis* it has been shown that peptidyl-prolyl isomerases PPIB as well as a ribosome-associated trigger factor are cold-induced or cold-accumulated proteins, respectively (Graumann *et al.* 1996; Graumann & Marahiel 1999a). This is in line with the observations reported for *E. coli*, according to which a mutant in the cold-induced trigger factor exhibits a cold-sensitive phenotype (Krandror & Goldberg 1997).

(c) *Cell differentiation*

Although *B. subtilis* has the ability to form highly resistant endospores upon nutrient deprivation or slow dehydration, even under optimal growth conditions this complex differentiation process requires time and reprogramming of protein biosynthesis (Stragier & Losick 1996). After a rapid temperature drop, it is therefore reasonable to expect that processes other than sporulation are required and are sufficient to cope with cold, as briefly described above. Although it has been demonstrated *in vitro* that KinC, one of the membrane-bound kinases

involved in the initiation of sporulation, is inhibited by UFAs that are produced by the cold-induced lipid desaturase Des (Strauch *et al.* 1992), it should be noted that, to our knowledge, no detailed examination concerning sporulation events during cold shock has yet been reported for this bacterium.

7. FUTURE PERSPECTIVES

To entirely understand the complex regulatory as well as functional organization of the *B. subtilis* cold shock stimulon, it is necessary to identify all of the involved players. Although the number of cold-induced proteins identified in *B. subtilis* (Graumann *et al.* 1996, 1997) is much greater than those characterized for *E. coli* (Jones *et al.* 1987), the examinations carried out so far were restricted to a rather narrow pI/MW range of between 4–7 and 5–100 kDa, respectively. Therefore, with the advent of modern DNA-array technologies, it will be one of the future goals to identify all cold-inducible genes at the transcriptional level in order to overcome the limitations of 2D gel electrophoresis and unravel those players which remained in the twilight zone until now. These are, most importantly, basic proteins, which comprise potential nucleic-acid-binding regulators as well as membrane components. Moreover, further analyses carried out using the appropriate deletion mutants will clearly reveal whether, for example, temperature perception systems such as DesKR are of global relevance during cold shock or not. Our alignments of the two-component sensor transduction system responsible for cold shock temperature perception in *Synechocystis* (Suzuki *et al.* 2000) suggest that *resDE*, *phoPR* and, most importantly, the essential two-component system encoded by the *yycFG* operon (Fabret & Hoch 1998; Fabret *et al.* 1999) rather than *desKR*, represent the primary candidates for a global temperature perception machinery in *B. subtilis* (data not shown). The existence of such additional membrane-bound sensor systems is also supported by the fact that overproduction of the DesR response regulator in the absence of DesK kinase allows for expression of *des* (Aguilar *et al.* 2001), which indicates cross-activation of DesR, possibly by another kinase. A third potential player might be the chemotaxis apparatus since in *E. coli* its associated two-component systems have been shown to function as temperature sensors (Nishiyama *et al.* 1999), and CheY, as part of the chemotaxis machinery, is a cold-induced protein in *B. subtilis* (Graumann *et al.* 1996).

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Discussion

M. Knight (Department of Plant Sciences, University of Oxford, Oxford, UK). A couple of questions about DesK. Do you have any feeling for how the temperature actually regulates the DesK activity?

M. A. Marahiel. Actually the group of Mendoza published, at the beginning of this year (2001) in the *EMBO Journal*, some data on the molecular thermosensing of

DesK. DesK, a histidine kinase that is membrane associated phosphorylates the response regulator DesR upon downshift in temperature. DesR-phosphate then acts as a positive regulator for the desaturase gene, therefore, inducing the synthesis of UFAs needed at low temperatures. These findings on how the two-component system DesK/DesR responds to temperature are based mainly on genetic experiments. It is not easy to express the DesK protein because it is very hard to express a membrane protein, and therefore no biochemical data are available on DesK as a kinase.

M. Knight. In the *Bacillus* genome are there other DesK like proteins that might be candidates for other operons?

M. A. Marahiel. There are about 35 two-component systems present in the *B. subtilis* genome and most of them are of unknown function, because not each system was disrupted to study the associated phenotype. As I told you, in our *desK* mutant we have analysed the transcriptional profiling, and compared it to the wild-type profile under low temperatures. We have discovered that actually the only gene that was not expressed in the *desK* mutant was the desaturase gene. So it seems that the two-component system DesK/DesR is very specialized for this case, I mean expressing the desaturase gene.

P. L. Davies (*Department of Biochemistry, Queen's University, Ontario, Canada*). You said that the substances are identified in solid bacteria. Are these found in places that experience freeze-thaw cycles?

M. A. Marahiel. Yes.

P. L. Davies. Does it have an antifreeze mechanism or something that protects it?

M. A. Marahiel. Yes. *B. subtilis* is a soil bacterium that lives in the upper part of the soil and interacts with plants and can also live on plant leaves, so this is the terrestrial region in which *B. subtilis* is found. The first experiment we have performed, a few years ago, was disrupting the genes encoding the major cold shock proteins (CspB, C and D). Upon disrupting the first gene we found that the *cspB* mutant was sensitive to freezing at -20°C when compared to wild-type cells that were adapted to low temperatures (10°C) before freezing. Cell viability in the *cspB* mutant was reduced to ca. 30–50%. At that time we thought that major CSPs may have an antifreeze function. However, based on the recent data on the interaction of the CSPs with nucleic acids and possibly the translation machinery, we think that they have a different function.

I. A. Shanks (*Colworth Laboratory, Unilever Research, Bedford, UK*). I just wondered whether you saw any similarity between the β -barrel-folded regions found in the CSPs and the β -pleated regions, the antigen-specific regions in immunoglobulin. And could it be some form of evolutionary precursor of the immune system?

M. A. Marahiel. The immunoglobulin fold also consists of three antiparallel β -strands at one side backed with two antiparallel β -strands from the other side. However, it does not look similar to the CspB fold because you have a different loop structure in the immunoglobulin fold that

has the antigen binding residues. And the immunoglobulin fold has a disulphide bond that is missing in the CspB structure. We do not see this in the CspB fold, but this nucleic acid-binding fold is based on a comparison of many cold shock proteins. This suggests that the CspB fold is new and may bind single-stranded DNA or RNA. It has no structural homology with other known proteins. Actually, when we published this work we just said this was a new fold and it was accepted as that.

By binding to single-stranded DNA or RNA, one would perhaps think that CSPs stabilize single-stranded DNA during transcription. Alternatively, CSPs might act as RNA chaperones to prevent formation of RNA secondary structures at low temperatures to facilitate translation. This idea appears to be supported by the recent discovery of co-localization of CSPs with ribosomes.

D. K. Hinch (*Max-Planck-Institut für Molekulare Pflanzenphysiologie, Potsdam, Germany*). Everything you describe was cold shock, that is, you make quick transition and then you look at what is happening. Are the same proteins continuously expressed if you look over hours or days or do other adaptive mechanism kick in, and is this just an emergency response?

M. A. Marahiel. These major CSPs are transiently induced after a sudden cold shock. We use these artificial conditions to define proteins made under those conditions. But also after a long period of growth at 15°C these proteins can be detected. And you can also see other proteins associated with osmoregulation, also induced under these prolonged conditions of growth at 15°C .

D. K. Hinch. So, they are also necessary for growth under low temperatures in the long run.

M. A. Marahiel. Yes.

GLOSSARY

CIP: cold-induced protein

CSP: cold shock protein

CSR: cold shock response

Dps: DNA protection during starvation protein

FA: fatty acid

IF1: translation initiation factor 1

(p)ppGpp: guanosine 5'-triphosphate-3'-diphosphate and guanosine 5'-diphosphate-3'-diphosphate

HSR: heat shock response

HU: collective term for the histone-like family of prokaryotic DNA-binding proteins (encoded by *hupA* and *hupB* in *E. coli*)

pI: isoelectric point

Lk: linking number

MW: molecular weight

SASP: small acid-soluble spore protein

SMC: chromosome partition protein

ΔT : temperature difference

Tw: twist

Wr: writhe

UFA: unsaturated fatty acid