

## Bacterial Adaptation to Cold

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### ABSTRACT

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The temperature downshift results in (1) decrease in membrane fluidity (2) changes in nucleic acid structures (3) reduced ribosome function (4) inefficient protein folding. Bacteria respond to this low temperature change by producing cold-shock proteins (CSPs) that bind to single-stranded RNA and DNA thereby, maintaining cell physiology. CSPs have been widely studied in *Escherichia coli* and *Bacillus subtilis*. Here we discuss the cold adaptation by desaturase system, RNA chaperone, and transcription antitermination function of CspA homologues.

### Introduction

One of the most frequent stresses faced by living organisms is change in temperature. Fluctuations in temperature have widespread effects on the growth and survival of bacteria, which have therefore developed mechanisms that allow them to adapt to these changes. One such mechanism adopted by bacteria is the cold shock response which consists of a number of adaptive changes ranging from alterations in membrane composition to alterations in the global protein profile of the cell (Phadtare, 2004). The cold shock response of a bacterium like *Escherichia coli* manifests itself as an acclimation phase during which cell growth completely (but temporarily) stops immediately after the temperature downshift. During this phase, numerous cold shock proteins are produced which help the cells to adopt to low

temperature. After the acclimation phase is over, the synthesis of most of the cold shock proteins decreases to a new basal level and synthesis of non-cold shock proteins resumes, allowing cells to grow at low temperature, although at a slower rate (Ermolenko and Makhataдзе, 2003).

Some bacteria such as *B. subtilis* and *Lactobacillus lactis* do not exhibit a growth cessation upon cold-shock response but instead immediately proceed to slow-rate low-temperature growth (Weber and Marahiel 2003). The chief consequences of temperature downshift include (1) decrease in membrane fluidity affecting membrane-associated cellular functions such as active transport and protein secretion (2) changes in nucleic acid structures (3) reduced ribosome function

which affects translation especially of non-cold-shock proteins and (4) inefficient protein folding. Various mechanisms are adapted by the cell to restore the flexibility of membranes for example, increasing proportion of unsaturated fatty acids (UFAs) (Phadtare and Severinov, 2010).

Temperature drop renders the ribosomes nonfunctional in translation of most cellular mRNAs. However, this effect is not as severe for mRNAs of genes encoding cold-shock proteins. This selective behaviour is due to structural elements present in cold-shock proteins mRNAs that promote translation initiation at low temperature. After the temperature downshift during the lag period, cold-shock ribosomal factors such as RbfA and CsdA are produced. These proteins bind the ribosomes and convert them to cold-adapted ribosomes that are capable of translating non-cold-shock mRNAs (Jones and Inouye, 1996).

### **Structure and function of CSPs**

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 (Wolffe *et al.*, 1992). The cold shock domain harbours the nucleic acid binding motifs, RNP-1 and RNP-2 (Landsman, 1992). CSPs, with a length of *ca.* 70 amino acids, share a highly similar overall fold. This fold consists of five antiparallel sheets, forming a  $\beta$ -barrel structure, with surface exposed aromatic and basic residues (RNP-1 and RNP-2 motifs on 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 (Lopez and 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 and Marahiel, 1998). 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).

### **Role of cold shock proteins in RNA modulation**

The different types of proteins that restructure RNAs are: (1) RNA chaperones that melt misfolded RNA molecules and thus promote proper folding (2) RNA annealers that accelerate annealing of complementary RNAs (3) RNA helicases that resolve the RNA structures using ATP hydrolysis and (4) specific RNA binding proteins that can also contribute to RNA folding by stabilizing specific RNA structures (Rajkowitsch *et al.*, 2007).

### **Adaptation of the cell membrane**

A rapid temperature downshift can induce phase separation of cell membrane phospholipids, and, as a result, there is a decrease in membrane fluidity and an increase in permeability (Cao-Hoang *et al.*, 2010). The membrane of Gram-negative cells is composed of lipopolysaccharides (LPS), which consist of a distal polysaccharide (O-antigen), and a core polysaccharide and lipid A. *E. coli* lipid A consists of two glucosamines with attached acyl chains (fatty acids); laurate is the fatty acyl chain usually detected in the cells growing at 37 °C. At low temperatures, there is a decrease in laurate counterbalanced by the appearance of

palmitoleate (Carty *et al.*, 1999). Effect of low temperature is counteracted by palmitoleate, an unsaturated fatty acid which increases membrane fluidity and lowers phase transition temperature in contrast to laurate. In *E. coli*, the cold-induced acyltransferase LpxP is responsible for attaching palmitoleate to lipid A upon temperature downshift (Vorachek-Warren *et al.*, 2002).

In *Bacillus subtilis*, adaptation of membrane fluidity involves rapid desaturation of fatty acids in already existing phospholipids. This happens by induction of fatty acid desaturase (Des), regulated by the sensor kinase DesK and the response regulator DesR (Aguilar *et al.*, 2001). The transmembrane domain of DesK acts as sensor of membrane fluidity (Albanesi *et al.*, 2004). A shift to lower temperature causes a decrease in membrane fluidity, which favours the DesK kinase state; DesK phosphorylates the transcriptional activator DesR which subsequently binds to the promoter of the *des* gene and activates synthesis of the D5-desaturase. This enzyme catalyses the introduction of a double bond into pre-existing fatty acid tails of phospholipids inside the cell membrane (Aguilar *et al.*, 2001; Albanesi *et al.*, 2004).

### **Regulation at the level of transcription**

The *cspA* transcript undergoes a 4-5-fold increase upon cold shock as revealed by studies using reporter gene fusions the reporter genes (Goldenberg *et al.*, 1997; Mitta *et al.*, 1997). No additional factors (other than cold) are necessary for *cspA* induction. The elements that enhance the level of *cspA* promoter activity include (1) an AT-rich sequence (UP element) immediately upstream of the -35 region (Goldenberg *et al.*, 1997; Mitta *et al.*, 1997) (2) an extended -10 box a TGn motif preceding the -10 box. However, none of these elements seem to specifically contribute to low-temperature activity of the

*cspA* promoter. The *cspA*, *cspB*, *cspG* and *cspI* genes possess a long 5' untranslated region (5'-UTR) which contains a highly conserved, 11-base sequence termed the 'cold box'. At high concentration CspA protein binds the cold-box and thus regulates its own expression. The overproduction of 5'UTR leads to prolonged synthesis of CspA, an effect suppressed by co-overproduction of CspA (Jiang *et al.*, 1996; Fang *et al.*, 1998)

Although CspA is also produced at 37°C during early exponential growth phase. This induction results from the location of the *cspA* gene near the *oriC* replication origin, which leads to higher gene dosage and higher stability of *cspA* mRNA due to lower RNase activity at this stage of growth (Brandi *et al.*, 1998). Production of CspA is observed at 37°C upon nutritional upshift (Yamanaka and Inouye 2001).

### **mRNA stabilization**

The *cspA* mRNA is stabilized immediately after cold shock. This stabilization is likely the major factor that leads to dramatic induction of CspA at low temperature (Phadtare and Severinov, 2005). The 5'-UTR was shown to be responsible for the extreme instability of *cspA* mRNA at 37°C (a half-life of 12 s) and has a positive effect on its stabilization upon cold shock (a half-life of more than 20 min) (Mitta *et al.*, 1997). The *cspA* promoter is active at 37°C, but the steady-state levels of *cspA* RNA and CspA synthesis are low at this temperature due to extreme instability of its mRNA. Using enzymatic and chemical probing, it was recently shown that the *cspA* mRNA undergoes a temperature-dependent structural rearrangement at low temperature, likely resulting from stabilization of an otherwise thermodynamically unstable folding intermediate. The "low temperature" structure is more efficiently translated and somewhat

less susceptible to degradation than the 37°C structure (Giuliodori *et al.*, 2005).

### **CspA homologs as transcription antiterminators**

At low temperature stable secondary structures of RNA are formed that interferes with both transcription and translation elongation. CspA homologs act as RNA chaperones due to their ability to 'melt' the secondary structures in nucleic acids and thus facilitating transcription and translation at low temperature. The function of CspA homologs as RNA chaperones promoting transcription antitermination has been studied in considerable detail (Phadtare *et al.*, 2002). Modulation of transcription termination by RNA-binding proteins involves resolving hairpin structures in nascent RNA that can act as transcriptional terminators or pause sites, thus leading to transcript elongation (Stulke 2002). The three-dimensional structures of CspA from *E. coli* and CspB from *B. subtilis* have been resolved by X-ray crystallography and NMR-analysis and found to be very similar (Feng *et al.*, 1998). The protein consists of five antiparallel  $\beta$ -strands ( $\beta$ 1 to  $\beta$ 5) that form a  $\beta$ -barrel structure with two  $\beta$ -sheets. Two RNA-binding motifs, RNP1 and RNP2, are located on the  $\beta$ 2 and  $\beta$ 3 strands, respectively. The proteins have an overall negative surface charge with positively charged amino acids surrounding a surface-exposed aromatic patch. After the initial approach to a RNA molecule through electrostatic attraction and subsequent binding through stacking of the aromatic RNP side chains with RNA bases, further intramolecular or intermolecular base pairing by a segment of RNA bound to a Csp protein is prevented by charge repulsion (Graumann and Marahiel 1998). CspA and its homologs do not exhibit high degree of specificity for their RNA/ DNA substrates (Lopez *et al.*, 2001). The RNA chaperone activity allows

the CspA homologs to act as transcription antiterminators and thus aid in cold acclimation of cells (Phadtare *et al.*, 2002)

The genes *malE* and *malK* (membrane related functions), *mopA* and *mopB* (chaperones), *dps*, *katG*, *rpoS*, *uspA* (stress response) showed firm dependence on Csp proteins for their expression at 15°C (Phadtare *et al.*, 2006).

### **Helicases and Exoribonucleases**

#### **SrmB and CsdA, the DEAD-box helicases involved in cold shock acclimation**

The DEAD-box RNA helicase family proteins play important roles in many cellular processes such as processing, transport or degradation of RNA or ribosome biogenesis (Iost and Dreyfus 2006). The *E. coli* encodes five DEAD-box RNA helicase family proteins, CsdA, DbpA, RhlB, RhlE and SrmB (Linder *et al.*, 1989) SrmB and CsdA are briefly described below.

#### **SrmB**

SrmB is involved in ribosome biogenesis. Deletion of *srmB* results in (1) slow-growth phenotype at low temperature (2) deficit in free 50S ribosomal subunits and (3) accumulation of a new ribosomal particle sedimenting around 40S. Thus, it was suggested that there is a step of 50S assembly, which involves a structural rearrangement that, at least at low temperature, requires SrmB (Charollais *et al.*, 2003).

#### **CsdA**

CsdA has been assigned multiple cellular functions including ribosome biogenesis, translation initiation and degradation of mRNAs. It was shown that CsdA is also involved in the biogenesis of the 50S

ribosomal subunits (Phadtare *et al.*, 2002) and associates with 50S precursors at low temperature (Charollais *et al.*, 2004). CsdA is homologous to eukaryotic translation initiation factor eIF4A. EIF4A catalyzes ATP-dependent unwinding of RNA duplexes and stimulates translational initiation. It was thus suggested that CsdA too may be involved in assisting the translation by promoting translation initiation of structured mRNAs (Lu *et al.*, 1999). The CsdA play a key role in mRNA degradation (1) is found in degradosomes in cold-adapted Cultures (2) is involved in efficient and selective degradation of Csp mRNAs by unwinding the mRNA secondary structure that impedes the processive activity of PNPase (Yamanaka and Inouye 2006). The helicase activity of CsdA is pivotal for promoting the degradation of mRNAs stabilized at low temperature: helicase-deficient CsdA mutants do not complement cold sensitivity of the *csdA* deletion cells (Awano *et al.*, 2007)

CsdA and SrmB share several properties: they (1) unwind nucleic acid duplexes with 3' or 5' extensions (2) stabilize certain mRNAs (3) bind to RNase E88 (4) participate in 50S assembly probably by modulating RNA structures through their unwinding activity and (5) act as RNA chaperones that prevent misfolding (Iost and Dreyfus 1994)

### **Cold-inducible exoribonucleases**

#### **RNase R**

PNPase, RNase R and RNase II are the three major 3'-to-5' processing exoribonucleases. These enzymes are primarily involved in RNA metabolism. Both PNPase and RNase R95 are induced by cold shock and are suggested to be the universal degraders of structured RNA in the cell (Cheng and Deutscher 2003). In *Bacillus subtilis* it was shown that cold-induced helicases and Csp

work in together to rescue misfolded mRNA molecules and maintain proper initiation of translation at low temperatures (Hunger *et al.*, 2006)

#### **PNPase**

PNPase is encoded by the *pnp* gene (Reiner 1969) and its expression is post-transcriptionally autoregulated at the level of both translation and mRNA stability (Jarrige *et al.*, 2001). PNPase binds to the 5' end of RNase III-processed *pnp* transcript which leads to the inhibition of translation and channeling of *pnp* mRNA into degradation pathway. During the cold acclimation phase this regulation is temporarily relieved leading to stabilization of the *pnp* mRNA and thus makes it extremely abundant (Briani *et al.*, 2007). PNPase is one of the main exoribonucleases in the cell and it promotes processive degradation of RNA (Mohanty and Kushner 2003).

### **Metabolism of RNA at Low Temperature**

At low temperature the RNA metabolism may be different than that at 37°C and it requires (1) the assistance of proteins that destabilize RNA secondary structures making them accessible to ribonucleases and (2) cold-inducible ribonucleases that can carry out RNA degradation efficiently and selectively to allow cell growth at low temperature. Note that both CsdA and PNPase are essential only at low temperature. Interestingly, these two proteins seem to function independently of each other as they cannot complement each other's functions (Phadtare and Konstantin Severinov 2010).

Temperature is one of the main variable environmental factors potentially impacting viability, and dealing with temperature change is crucial for adaptation. Important changes in RNA metabolism help survival in



the cold. Cells produce two main groups of cold shock proteins, which modulate the RNA secondary structures and thus play important role(s) in the cold-shock adaptation of cells. Elucidation of the mode of action of various cold shock proteins on each target is necessary for complete understanding of cellular adaptation to low temperatures.

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