

## Review

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# Bacterial adaptation to cold

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Micro-organisms react to a rapid temperature downshift by triggering a physiological response to ensure survival in unfavourable conditions. Adaptation includes changes in membrane composition and in the translation and transcription machineries. The cold shock response leads to a growth block and overall repression of translation; however, there is the induction of a set of specific proteins that help to tune cell metabolism and readjust it to the new conditions. For a mesophile like *E. coli*, the adaptation process takes about 4 h. Although the bacterial cold shock response was discovered over two decades ago we are still far from understanding this process. In this review, we aim to describe current knowledge, focusing on the functions of RNA-interacting proteins and RNases involved in cold shock adaptation.

## Introduction

Micro-organisms have to adapt constantly to different environmental changes. These include nutrient and oxygen availability, osmotic stress and changes in temperature. After a temperature decrease, a number of important changes occur in cellular physiology, such as a decrease in membrane fluidity and stabilization of the secondary structures of nucleic acids, which leads to a reduced efficiency of RNA transcription, translation and degradation (Phadtare, 2004). The cold shock response enables the cell to counteract these unfavourable changes, mostly by the selective production of a specific set of proteins (cold-induced proteins) (Table 1).

In laboratory conditions, the cold shock response is triggered by an abrupt shift of a culture growing exponentially from its optimum temperature to a lower temperature (usually 37 °C to 15 °C). Upon temperature downshift, there is a transient arrest of cell growth for 3 to 6 h – this period is termed the acclimation phase. In the acclimation phase the production of most proteins stops, except for cold-inducible proteins, which are still translated (Polissi *et al.*, 2003). After the acclimation phase, cells become adapted to low temperature and resume growth but at a slower rate (Phadtare, 2004), the expression of the cold-inducible proteins declines and bulk protein synthesis restarts (Fig. 1).

## Adaptation of the cell membrane

A rapid temperature downshift can induce phase separation of cell membrane phospholipids, and, consequently, there is a decrease in membrane fluidity and an increase in permeability (Fig. 2) (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). In contrast to laurate, palmitoleate is an unsaturated fatty acid. The presence of palmitoleate increases membrane fluidity and lowers its phase transition temperature, counteracting the effect of low temperature. 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 trans-membrane domain of DesK was described as a probable sensor of membrane fluidity (Albanesi *et al.*, 2004). A lower temperature causes a decrease in membrane fluidity, which favours the active state of the DesK kinase; 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).

## Adaptation of RNA metabolism

Most of the cold-inducible proteins are involved in RNA metabolism, for example the RNA helicase DeaD and two exonucleases RNase R and PNPase. The proteins most strongly induced are nucleic acid chaperones from a family

**Table 1.** Cold-inducible proteins

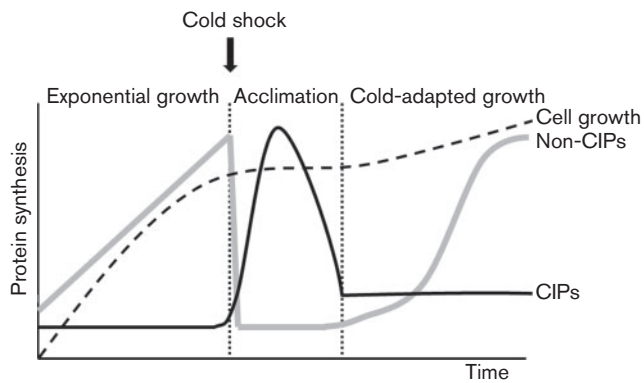
The table shows gene names, and the functions of the gene products.

Gene	Description/function in cold shock	References
<i>aceE</i>	Pyruvate dehydrogenase, decarboxylase	Gualerzi <i>et al.</i> (2003)
<i>aceF</i>	Pyruvate dehydrogenase, dihydrolipoamide acetyltransferase	Gualerzi <i>et al.</i> (2003)
<i>cspA</i>	Cold-inducible RNA chaperone and anti-terminator; transcriptional enhancer	Gualerzi <i>et al.</i> (2003)
<i>cspB</i>	Cold shock-inducible; function unknown	Gualerzi <i>et al.</i> (2003)
<i>cspE</i>	RNA chaperone; transcriptional antitermination	Gualerzi <i>et al.</i> (2003)
<i>cspG</i>	Cold shock protein homologue, cold-inducible; function unknown	Gualerzi <i>et al.</i> (2003)
<i>cspI</i>	Cold shock protein, cold shock-inducible; function unknown	Gualerzi <i>et al.</i> (2003)
<i>deaD</i>	ATP-dependent RNA helicase, facilitates translation of mRNAs with 5' secondary structures	Jones <i>et al.</i> (1996); Moll <i>et al.</i> (2002)
<i>dnaA</i>	DNA binding and replication initiator, global transcription regulator	Gualerzi <i>et al.</i> (2003)
<i>gyrA</i>	DNA gyrase, subunit A; DNA binding/cleaving/rejoining subunit of gyrase	Gualerzi <i>et al.</i> (2003)
<i>hns</i>	Nucleoid protein, transcriptional repressor, repressor supercoiling	Gualerzi <i>et al.</i> (2003)
<i>hscA</i>	DnaK-like chaperone	Lelivelt & Kawula (1995)
<i>hscB</i>	DnaJ-like co-chaperone for HscA	Lelivelt & Kawula (1995)
<i>hupB</i>	Nucleoid protein, DNA supercoiling	Giangrossi <i>et al.</i> (2002)
<i>infA</i>	Protein chain initiation factor IF1, translation initiation	Gualerzi <i>et al.</i> (2003)
<i>infB</i>	Protein chain initiation factor IF2, translation initiation, fMet-tRNA binding, protein chaperone	Gualerzi <i>et al.</i> (2003)
<i>infC</i>	Protein chain initiation factor IF3, translation initiation, stimulates mRNA translation	Gualerzi <i>et al.</i> (2003)
<i>lpxP</i>	Lipid A synthesis; cold-inducible	Carty <i>et al.</i> (1999); Vorachek-Warren <i>et al.</i> (2002)
<i>nusA</i>	Transcription termination/antitermination/elongation L factor	Bae <i>et al.</i> (2000)
<i>otsA</i>	Trehalose phosphate synthase; cold- and heat-induced, critical for viability at low temperatures	Kandror <i>et al.</i> (2002)
<i>otsB</i>	Trehalose phosphate phosphatase; cold- and heat-induced, critical for viability at low temperatures	Kandror <i>et al.</i> (2002)
<i>pnp</i>	3'-5' exoribonuclease; component of RNA degradosome; cold shock protein required for growth at low temperatures	Yamanaka & Inouye (2001)
<i>rnr</i>	3'-5' exonucleases; increases 10-fold in cold shock	Cairrão <i>et al.</i> (2003)
<i>rbfA</i>	Ribosome-binding factor required for efficient processing of 16S rRNA; cold shock adaptation protein	Gualerzi <i>et al.</i> (2003)
<i>recA</i>	General recombination and DNA repair; induction of the SOS response	Dammel & Noller (1995); Gualerzi <i>et al.</i> (2003)
<i>tig</i>	Protein-folding chaperone, multiple stress protein, ribosome-binding	Kandror <i>et al.</i> (2002); Kandror & Goldberg (1997)
<i>ves</i>	Cold- and stress-inducible protein, function unknown	Yamada <i>et al.</i> (2002)
<i>yfiA</i>	Protein Y, associated with 30S ribosomal subunit, inhibits translation	Di Pietro <i>et al.</i> (2013)

of cold shock proteins termed Csp. This suggests that tuning RNA metabolism is crucial for adaptation.

Low temperature causes stabilization of the secondary structures of nucleic acids and this can impair transcription, RNA degradation and translation. The main function of the cold-induced proteins involved in RNA metabolism is to prevent secondary structure formation or to facilitate degradation of structured RNA. Proteins from the Csp family are nucleic acid chaperones and this activity prevents the formation of secondary structures. Helicase DeaD can melt secondary structures and facilitate their degradation by the cold shock exoribonucleases PNPase and RNase R. RNase R is the only 3-5' exonuclease in *E. coli* that efficiently degrades double-stranded RNA (Matos *et al.*, 2009; Phadtare, 2011).

**Transcription.** At low temperatures the curvature of DNA changes, becoming more negatively supercoiled. This effect partially depends on the activity of the histone-like HU protein (HupB) and gyrase (GyrA), an enzyme capable of introducing negative supercoils. The effect is transient and remains for about an hour, during the acclimation period (Mizushima *et al.*, 1997). The increase in negative supercoiling can in turn trigger the transcription of cold-induced genes in a way that is not completely understood. Studies in *Synechocystis* sp. showed that some cold-induced genes were repressed by the presence of a DNA gyrase inhibitor (Prakash *et al.*, 2009). A converse effect is observed upon heat shock: the DNA relaxation triggered by temperature increase also induces the expression of a specific set of genes (López-García & Forterre, 2000).



**Fig. 1.** Cold shock adaptation process. After cold shock induction, cell growth (dashed line) stops for several hours (acclimation phase). During acclimation, bulk protein synthesis stops (non-CIPs grey line), while the small fraction of cold-induced proteins (CIPs) important for adaptation is expressed (black line). After the acclimation phase, growth and protein production resumes although at slower rate, and most of the CIPs are inhibited. (Adapted from Horn *et al.*, 2007).

The majority of the proteins from the Csp family are capable of binding to DNA (Phadtare & Inouye, 1999; Yamanaka *et al.*, 1998). Cold-induced Csp have been functionally linked to the maintenance of chromosome structure (Chaikam & Karlson, 2010). CspE overexpression can promote or protect chromosome folding (Hu *et al.*, 1996).

CspA and CspE were shown to be involved in transcription at low temperatures, acting as transcription antiterminators, preventing hairpin formation that may lead to premature termination (Bae *et al.*, 2000). Another protein with anti-terminator function which is induced during cold is NusA (Bae *et al.*, 2000).

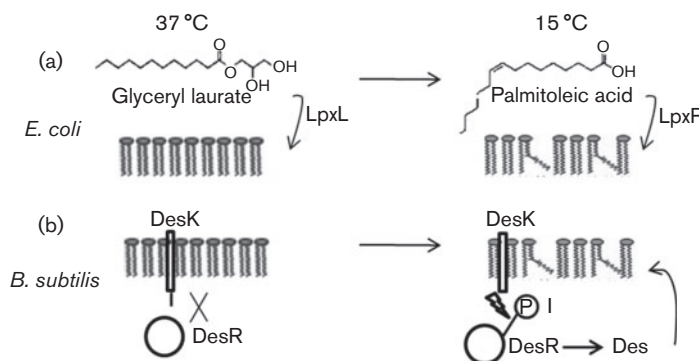
The transcription factor RpoS (also known as  $\sigma$ S) is not a cold-induced protein but it has been connected with the

expression of genes in response to many stresses, including the shift to low temperature. In a study focusing on *E. coli* biofilm formation at low temperatures, 40% of the genes preferentially expressed at 23 °C were shown to be controlled by RpoS (White-Ziegler *et al.*, 2008). Of these genes, several are implicated in biofilm development and an RpoS mutation decreases biofilm formation at 23 °C (White-Ziegler *et al.*, 2008).

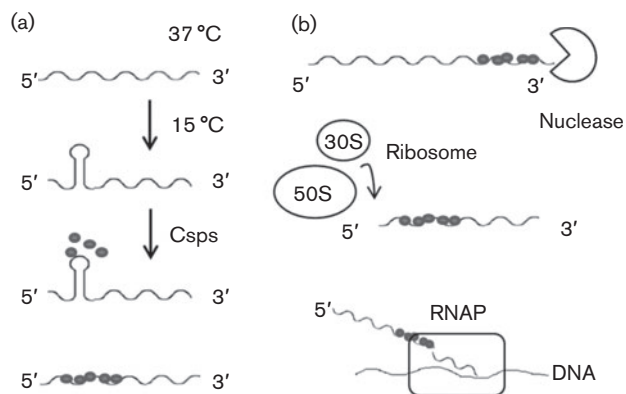
**Degradation/stabilization.** Several proteins from the Csp family are implicated in RNA stabilization and/or degradation. The chaperone functions of the Csp are crucial for mRNA stability in cold conditions. CspA, the major cold-induced protein of this family, destabilizes secondary structures and functions as a chaperone to maintain RNA in a single-stranded state, which favours its degradation (Fig. 3). CspE acts in the opposite way, hampering RNA degradation. CspE was shown to bind poly-A tails, interfering with their digestion by PNPase, and can also inhibit RNA internal cleavage by RNase E (Feng *et al.*, 2001).

In *E. coli*, DeaD is a DEAD-box helicase which under cold conditions is incorporated into the main RNA degrading machine, the degradosome. Its helicase activity probably helps to degrade structured RNAs. *In vitro* DeaD directly associates with RNase E – the main endonuclease and the scaffold of the degradosome complex (Prud'homme-Généreux *et al.*, 2004). RhlB – the other degradosome associated helicase – is still detected in the cold shock adapted degradosome, suggesting that either both helicases associate with RNase E (Khemic *et al.*, 2004) or cold shocked cells have a heterogeneous population of degradosomes (Prud'homme-Généreux *et al.*, 2004).

The helicase activity of DeaD is also involved in ribosome biogenesis in low temperature conditions. DeaD deletion leads to the depletion of the mature 50S ribosomal subunit and accumulation of the 40S-like particle (Charollais, 2004). RbfA is implicated in the maturation of the 30S



**Fig. 2.** Adaptation of the cell membrane to the cold. (a) In *E. coli*, at 37 °C LpxL is responsible for attaching laurate onto the lipid A that is present in the lipopolysaccharides of the cell membrane. At low temperatures, there is a decrease in laurate, counterbalanced by the appearance of palmitoleate. LpxP is a cold-induced acyltransferase which is responsible for attaching palmitoleate to lipid A. (b) In *B. subtilis*, upon a drop in temperature, the membrane protein DesK mediates phosphorylation of DesR which results in transcriptional activation of *des*. Activation of this gene leads to Des protein synthesis; Des desaturates the acyl chains of pre-existing membrane phospholipids.



**Fig. 3.** Role of the Csp proteins in adaptation to low temperature. (a) At low temperature RNA structures become more stable, Csp (spheres) can melt double-stranded RNA, and stabilize single-stranded RNA conformation. (b) New secondary structures can impair RNA metabolism at different stages; degradation – by hampering progress of the nucleases; translation – by preventing the formation of translation initiation complex by ribosome subunits; transcription – by causing premature termination of RNA polymerase (RNAP). The chaperone activity of Csp prevents these undesirable effects.

subunit and is also induced at low temperatures (Dammel & Noller, 1995).

PNPase, one of the main *E. coli* exonucleases, increases its level about twofold upon cold induction and is important for cell survival at lower temperatures (Yamanaka & Inouye, 2001). PNPase helps to repress the production of the Csp family of proteins at the end of the acclimation phase. In strains lacking PNPase, poly-A polymerase (PAP) or DeaD helicase the cells have normal induction of Csp; however, these strains have a high level of Csp even after 24 h, indicating that these proteins are contributing to the specific degradation of Csp mRNAs (Yamanaka & Inouye, 2001). PNPase RNase activity was shown to be critical in cold conditions and its activity is complemented by RNase II (Awano *et al.*, 2008).

Of the three main exoribonucleases in *E. coli*, PNPase, RNase II and RNase R, only RNase R suppresses a DeaD deletion phenotype at low temperatures (Awano *et al.*, 2007; Phadtare, 2012). RNase R is a cold shock protein, increasing its level about 10-fold under cold conditions (Andrade *et al.*, 2009; Cairrão *et al.*, 2003). It is the only *E. coli* exonuclease able to processively digest RNA secondary structures without the help of a helicase. The ability to unwind secondary structures was shown to be dependent on RNase R cold shock domains (CSDs) (Awano *et al.*, 2010) and is essential for the suppression of the phenotype associated with DeaD deletion. At low temperatures RNA structures are stabilized. The ability to degrade secondary structures is probably the main reason for the importance of RNase R in the cold.

**Translation.** As a result of a temperature downshift there is a general translation block, with the exception of the translation of the cold-inducible proteins. PY is one protein involved in the block in translation. PY reduces translation of some mRNAs due to its capacity to bind to 30S subunits. PY binding sequesters 30S and 50S subunits, favouring their association into idle 70S monomers. As a consequence the number of ribosomal subunits able to form 70S initiation complexes decreases. The PY protein is detected in the ribosomal fraction in the first hour after cold induction, remains through the acclimation phase and then is released when growth resumes. The association of PY with ribosomes was also detected during stationary phase at 37 °C (Di Pietro *et al.*, 2013).

Structured RNA can slow down the progression of the ribosomes and impair translation. Moreover, RNA structures can hide the Shine–Dalgarno sequences and inhibit translation initiation. Preventing the formation of RNA structures counteracts this effect. The Csp proteins together with RNA helicases like DeaD can improve translation by melting RNA structures.

The ribosome itself adapts to the new conditions. *In vitro* experiments proved that the *E. coli* translation apparatus in the cold can preferentially act on mRNAs from cold-induced genes (Giuliodori *et al.*, 2004). There are both *cis* elements (specific structures in the mRNAs of genes induced in the cold) and *trans* factors involved in this specific induction. The best known *trans*-acting factors include Csp proteins and translation initiation factors (IF1, IF2, IF3). The levels of IFs increase due to the cold shock translational bias (Gualerzi *et al.*, 2003). IF3 and IF1 levels seem to be especially important for translation of cold-specific genes. IF3 preferentially stimulates cold shock mRNA translation and IF1 enhances the effect of IF3 without influencing translational specificity (Gualerzi *et al.*, 2003).

Low temperature can cause protein mis-folding. IF2 and Hsc66 were suggested to be involved in correct protein folding in the cold (Caldas *et al.*, 2000; Lelivelt & Kawula, 1995). Another protein that plays an important role in co-translational protein folding is the trigger factor (TF). TF levels increase progressively as temperature drops. *E. coli* cells with reduced TF content are cold sensitive, while cells overexpressing this protein have an enhanced viability in low temperatures (Kandror & Goldberg, 1997). TF is thought to help protein synthesis and folding to continue in the cold and also to maintain pre-existing proteins in a functional form by promoting refolding of cold-damaged proteins (Phadtare, 2004). In *B. subtilis*, TF is also involved in protein folding at low temperatures (Graumann *et al.*, 1996).

### Mechanisms of induction of specific proteins in the cold

During adaptation to the cold, expression of most proteins is downregulated but cold-induced proteins are still

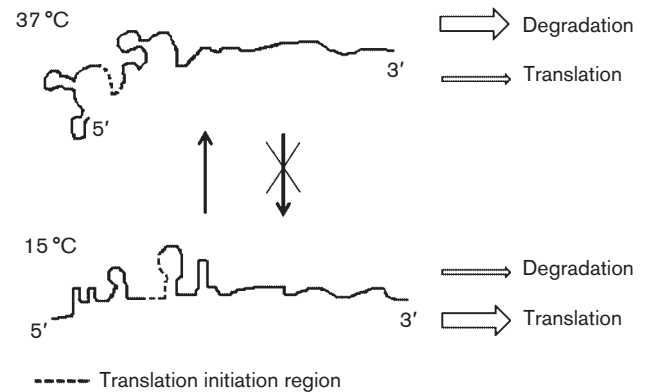
expressed or even induced. There are no specific transcription factors responsible for this induction and it seems that regulation relies more on changes in mRNA stability and accessibility to the translation machinery. CspA cold induction has been most extensively studied and it is known to be very highly regulated. In cold conditions, CspA accounts for almost 13 % of total cell proteins, while at 37 °C it is reduced to low levels (Goldstein *et al.*, 1990).

The *cspA* promoter responds to a temperature downshift (Goldenberg *et al.*, 1997; Mitta *et al.*, 1997). The core promoter of *cspA* can be induced by cold shock up to threefold at 15 °C. CspA RNA levels are also increased due to stabilization of the transcript (Fang *et al.*, 1997). The *cspA* mRNA is extremely unstable at 37 °C but highly stable at 15 °C, with a half-life increase from 12 s to 15 min, respectively (Fang *et al.*, 1997; Goldenberg *et al.*, 1997). After a temperature shift from 15 °C to 37 °C, around 99 % of the *cspA* mRNA is degraded after 1 min. These changes in stability are thought to be caused by *cspA* 5' untranslated region (5'UTR) structural rearrangements, which cause instability at 37 °C but have a positive effect on mRNA stabilization at low temperatures (Mitta *et al.*, 1997). Similarly, structural changes of the *cspA* 5'UTR impact the translation of this messenger by opening the translation initiation region (TIR) at low temperatures (Giuliodori *et al.*, 2010) (Fig. 4). Increased levels of CspA itself, as well as other translation initiation factors like IF3 and IF1, additionally act as *trans* factors which facilitate CspA production. (Giuliodori *et al.*, 2004).

### Cold shock studies in the context of industry and biomedical applications

Investigating the mechanism of cold adaptation may lead to the discovery of new agents that can selectively modulate the adaptation and growth of micro-organisms at low temperatures. Therefore, understanding cold shock adaptation has important applications in industry and health. Overexpressing Csp proteins in commercially used *Lactobacillus* strains can increase the viability of these micro-organisms in stationary phase or during freezing cycles (Derzelle *et al.*, 2003). Similar positive effects on viability are registered if bacteria are pre-incubated in the cold prior to freezing. This is due to the pre-induction of cold-specific proteins. A series of expression vectors (pCold) based on *cspA* transcript sequences has been proven of great utility to produce high levels of proteins in *E. coli* (Qing *et al.*, 2004). This is also the case of other expression vectors which use *cspA* promoter sequences (Vasina & Baneyx, 1996).

On the other hand, the deletion/repression of certain genes can decrease or prevent the proliferation of virulent micro-organisms under refrigerated conditions. This is the case for the human pathogen *Listeria monocytogenes*. A recent study showed that proteins from the Csp family play an important role in gene regulatory mechanisms. The proteins of this family, in particular CspB, regulate the



**Fig. 4.** CspA mRNA as a thermosensor. With a drop in temperature the 5'UTR of the *cspA* mRNA structure changes. The *cspA* 5'UTR rearrangement at low temperature stabilizes mRNA and ensures its efficient translation due to a more open translation initiation region. (Adapted from Giuliodori *et al.*, 2010.)

production of the virulence factor listeriolysin O (LLO). Deletion of the *cspA*, *cspD* and *cspB* genes, or the *cspB* gene alone, leads to cells with decreased expression of the *hly* gene (which codes for LLO protein synthesis). As a consequence, in these strains, *L. monocytogenes* cells become less virulent (Schärer *et al.*, 2013). The same applies in *Yersinia enterocolitica*, which requires the expression of *pnp* for growth at low temperatures (Goverde *et al.*, 1998).

### Conclusions

Fast adaptation to dynamically changing environments is the major reason for the evolutionary success of unicellular organisms. 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. Namely, there is the specific induction of RNA chaperones and there are changes in the levels of certain RNases. The investigation of the adaptation process of micro-organisms can provide important information with applications in industry and in health.

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