

Chapter 12

Proteomic Insights: Cryoadaptation of Permafrost Bacteria

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12.1 Introduction

Permafrost, which is defined as a subsurface frozen layer that remains frozen for more than 2 years, makes up more than 20% of the land surface of the earth, including 82% of Alaska, 50% of Russia and Canada, 20% of China, and most of the surface of Antarctica (Harris 1986; Williams and Smith 1989; Storad 1990). Permafrost poses unique challenges to its resident biota because of the permanently cold temperature of the soils, averaging -10 to -12°C , and the length of time over which the soils were frozen, which may be from a few thousand to even 2–3 million years.

To survive at subfreezing temperatures in permafrost, microbes have apparently developed various adaptive mechanisms. Electron microscopic examination of bacterial cells in a chip of permafrost core revealed that bacterial cells may survive due to reduction of cell size and formation of “dwarf” curved forms similar to nanoforms. The in situ permafrost bacteria, further characterized by thickened cell walls, altered structure of cytoplasm, compact nucleoid, showed similarities to cyst-like resting forms of non-spore-forming bacteria (Soina et al. 2004). The survival mechanisms may include reduction of the polar polysaccharide capsular layer, decrease of the fractional volume of cellular water, increase of the fraction of ordered cellular water, or extraction of energy by catalyzing redox reactions of ions in thin aqueous films in permafrost (McGrath and Gilichinsky 1994; Ostroumov and Siegert 1996; Mindock et al. 2001; Gilichinsky 2002). Among such adaptive processes, not only the bacteria themselves might be affected by environmental low temperature and induced cold-adapted features, but also the production of cold-induced organic molecules within them, such as polysaccharides, proteins and enzymes that sustain their metabolism at low temperatures.

Progress on low-temperature adaptation research has been achieved mainly through genomic or physiological studies. Proteomic analysis provides the dynamic information of cells which reflects the actual live status of cells. Protein patterns demonstrated that growth temperature substantially reprogrammed the proteome.

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Identification of all the proteins, including those differentially expressed under different conditions, will facilitate the understanding of the adaptation process. Comparative proteomic studies of various microorganisms during growth at different temperatures could be found (Sinchaikul et al. 2002; Goodchild et al. 2005; Kawamoto et al. 2007). Some of these differentially produced proteins displayed temperature trends: some proteins accumulated to high levels at low temperatures, while other protein expressions are elevated at high temperatures. Here we review the proteomic studies of cryoadaptation of permafrost bacteria.

12.2 Proteomic Studies of Low-Temperature Adaptations in Permafrost Bacteria

In the discussion of bacterial low-temperature adaptation, specific sets of cold-induced proteins (CIPs) have been considered to facilitate and allow cell growth at low temperature. CIPs are defined as proteins that are preferentially or uniquely present at low temperatures, and are thought to contribute specially to the ability of organisms to function at low temperatures (Fukunaga et al. 1999). CIPs could be further classified into cold-shock proteins (CSPs) and cold-acclimation proteins (CAPs). The term “CSPs” is used here for proteins that are transiently over-expressed after an abrupt shift to a low temperature, and the term “CAPs” is used for the proteins synthesized at a greater level during continuous growth at low temperatures as compared with high temperatures. CSPs and CAPs have been considered to facilitate and allow cell growth at low temperatures, and both sets of proteins may share functionality at both the molecular and cellular level (Whyte and Inniss 1992; Bayles et al. 1996; Berger et al. 1996; Panoff et al. 1997). Similarities between the CSPs and CAPs may suggest that these proteins are of significance to both shock recovery as well as constant growth in a new environment. The synthesis of CIPs in response to continuous growth at low temperatures in comparison to optimal growth temperature has been studied in two strains of the genus *Exiguobacterium* and two strains of the genus *Psychrobacter* isolated from Siberian permafrost and water brine samples (Table 12.1).

12.2.1 Cold-Inducible Proteins (CIPS)

The detection and identification of CIPs present during growth at 16°C, 4°C, and -4°C (salinity remained constant at 5%) by two-dimensional electrophoresis has been reported in *Psychrobacter cryohalolentis* K5 (Bakermans et al. 2007). Changes in the growth temperature regime differentially induce the synthesis of a large set of specialized proteins needed to maintain growth and reproduction at different temperatures. Twenty-eight of the CIPs were identified in *P. cryohalolentis* K5.

Table 12.1 List of permafrost strains studied by proteomics approaches

Strain	Origin (age)	Location, collection date	Environmental conditions	References
<i>E. sibiricum</i> 7-3 (VKM B 2374)	Alluvium loam and sandy loam (30,000 years)	Khomus-Yuryakh river; 68°19'N, 154°58'E; August 1989	8 m, -10°C, pH 7	Chong et al. (2000)
<i>E. sibiricum</i> 255-15 (DSM 17290)	Lake-alluvium loam and sandy loam (3 million years)	Bol'shaya Chykochnya river; 69°10'N, 158°4'E; July 1994	43.6 m, -10°C, pH 7.3	Qiu et al. (2006)
<i>P. arcticus</i> 273-4 (DSM 17307)	Alluvium sandy loam (30,000 years)	Malay Kon'kovaya river; 69°N, 158°30'E; August 1997	12.5 m; -10°C, pH 6.9	Zheng et al. (2007)
<i>P. cryohalolentis</i> K5 (DSM 17306)	Brine water lens within alluvial icy complex (43,000 years)	Lake Yakutskoe; 69°50'N, 159°30'E; August 1999	24 m, -11°C, pH 7.4, salinity 150 g·l ⁻¹	Bakermans et al. (2007)

Sample description was adopted from Gilichinsky et al. (2005) and Vishnivetskaya et al. (2000, 2006)

Among them, 15 proteins synthesized at 16°C were overexpressed at low temperatures, eight CIPs were detected during growth at both 4°C and -4°C, and five CIPs were specifically detected during growth at -4°C. These negative temperature-inducible proteins included:

The B subunit of F1/F0 ATP synthase, AtpF

The outer membrane efflux system protein, TolC

The elongation factor Ts, EF-Ts

A hypothetical protein with a bacterial Ig-like domain, Pcryo_1988, and

The outer membrane receptor for ferric citrate transport, FecA.

The drastic increase in relative abundance of these proteins at -4°C, relative to 4°C and 16°C, suggest specific stress on energy production, protein synthesis, and transport during growth at subzero temperatures. The efflux transporter TolC (as AcrAB-TolC) has a broad substrate range, and transports antibiotics, detergents, etc. suggesting an increased need to export potentially harmful molecules at -4°C.

12.2.2 Cold-Shock Proteins (CSPs)

CSPs comprise a family of small proteins that are structurally highly conserved, bind to single-stranded nucleic acids and are involved in a variety of cellular processes, such as transcription (Ermolenko and Makhatadze 2002). Bacterial

CSPs are rich in aromatic and basic amino acids, and their expression peak occurs shortly after a rapid temperature downshift to regulate the adaptation to cold stress, but they are also present under normal conditions to regulate other biological functions (Barbaro et al. 2002; Guo and Gong 2002). The cold-shock phenomenon was originally found in *Escherichia coli* at a temperature downshift from 37°C to 10°C (Jones and Inouye 1994), and was later found to be a cold-shock response common to many bacterial species (Kim et al. 1998b; Lottering and Streips 1995; Obata et al. 1998) some eukaryotes (Somer et al. 2002), and archaea (Cavicchioli et al. 2000). The major cold-shock protein CspA of *E. coli* has high sequence similarity with eukaryotic Y-box DNA-binding proteins that are known to be involved in regulation of several transcription and translation processes (Lee et al. 1994). A homolog of CspA was found to be upregulated following cold shock in psychrotrophic bacterium *Arthrobacter globiformis* SI55, but unlike its mesophilic counterparts, it was still expressed during prolonged growth at 4°C. The synthesis of this CspA-like protein was regulated at the translational level, and it was shown that growth resumption following a temperature downshift correlated with CspA expression (Berger et al. 1997). Similarly, psychroactive bacteria from permafrost showed overexpression of the CSPs during continuous low-temperature growth.

The presence of homologous cold-shock protein C (CspC, 7.255 kDa) in *Exiguobacterium sibiricum* 7-3 and three Csps (with Mr 7.150, 7.414 and 7.444 kDa) in *E. sibiricum* 255-15 was detected by high-performance liquid chromatography (HPLC) associated with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) (Chong et al. 2000; Qiu et al. 2006). Along with CspC, the overexpression of two other CSPs (CSP CSI4B 1,924.3 kDa, CSP CSI5 1,359.7 kDa) was observed in *E. sibiricum* 7-3 during low temperature growth (Chong et al. 2000). Three major CSPs from *E. sibiricum* 255-15 were homologous with 65.15%, 66.67%, and 59.09% sequence overlap to CspA in *E. coli*, and over 74% when compared to CspB, CspC, and CspD in *Bacillus subtilis* (Qiu et al. 2006). What is interesting is that unlike in *E. coli*, *B. subtilis*, and *E. sibiricum* 7-3 family of CSPs, those in *E. sibiricum* 255-15 were found similarly expressed at 25°C and 4°C, and represent about 10% of the total soluble proteins in cells grown at both temperatures. This result suggests that the genes for these proteins are turned on continuously to produce “shock” proteins to protect the cells from damage during abrupt changes in environmental conditions. Such behavior has been observed in other psychroactive bacterium such as *Psychrobacter arcticus* 273-4, where it has been shown that certain proteins (e.g. ribosomal proteins, ATP-dependent helicase, Elongation factor Ts) are always synthesized (Zheng et al. 2007). Apparently these organisms, which survive for long periods of time under extreme conditions, have adapted such a continuous expression as a means of survival. However, a putative CSP (8,111 Da/4.9 pI) was detected in *P. arcticus* 273-4 at 4°C and at both 22°C and 4°C when grown in medium with 5% NaCl, but was not detected at 22°C in ½ Tryptic Soy Broth (TSB) (Zheng et al. 2007). Another strain, *P. cryohalolentis* K5, showed the presence of CSP (CspA, 7.45 kDa) only at temperatures of 4°C and -4°C (Bakermans et al. 2007).

12.2.3 Cold-Acclimation Proteins (CAPs)

A set of proteins which are distinct from CSPs, and are specifically synthesized during continuous growth at low temperatures, are termed CAPs (Roberts and Inniss 1992; Whyte and Inniss 1992; Berger et al. 1996; Colucci and Inniss 1996). Recently, CAPs distinct from CSPs have been identified in the mesophilic bacteria *Enterococcus faecalis* during continuous growth at 8°C and *Listeria monocytogenes* at 10°C (Panoff et al. 1997; Liu et al. 2002).

From peptide analysis of the whole-cell lysates of *E. sibiricum* 255-15, 39 proteins with Mr ranging from 7 to 95 kDa were identified to be present at an increased level at the lower temperature and were considered to be CAPs, 16 of which were not detected at 25°C (Qiu et al. 2006). Some of these CAPs, such as trigger factor (TF) and pyruvate dehydrogenase, were characterized as CSPs in *E. coli* (Kandror and Goldberg 1997; Jones et al. 2006). TF in *E. coli* is a molecular chaperone with prolyl-isomerase activity, and associates with nascent polypeptides on ribosomes, binds to GroEL, enhances GroEL's affinity for unfolded proteins, and promotes degradation of certain polypeptides (Kandror and Goldberg 1997). TF levels increased progressively as growth temperature decreased and even rose in cells stored at 4°C. *E. coli* cells with reduced TF content die faster, while cells overexpressing TF showed greater viability. Thus, TF represents an example of an *E. coli* protein which protects cells against low temperatures. Unlike the TF, the role of pyruvate dehydrogenase has not yet been well-understood. Presumably, it is involved in the intensification of glycolysis and the suppression of the tricarboxylic acid cycle, i.e., in the processes that are observed upon the retardation of cell growth, and the adaptation of cells to stresses (Graumann and Marahiel 1996; Qiu et al. 2006).

The overexpression of heat-shock protein 70 (Hsp70) molecular chaperones was observed in *E. sibiricum* 255-15 during the cold-adaptation process. The heat-shock proteins may function as molecular chaperones that play an important role in protein folding, and — like DnaK — have functions in refolding of misfolded proteins that are essential under stress. Thus, these so-called “heat-shock proteins” are not simply heat-shock-specific proteins. They should more appropriately be called “temperature-stress proteins” (Qiu et al. 2006). While Hsp70 of *P. articus* 273-4 was overexpressed only in response to low temperature, chaperonin Hsp60 was found to be induced by low temperature or salt, where it was down-regulated if both of these extremes were present (Zheng et al. 2007).

Chaperone proteins DnaK and GroEL were found to be actively synthesized in response to heat, cold, and chemical stress (Salotra et al. 1995; Phan-Thanh and Gormon 1997). The phage-shock protein A (PspA) of *E. sibiricum* 255-15 was the highest overexpressed protein at low growth temperatures, whose expression ratio was over 70 (Qiu et al. 2006). Presently, the exact function of PspA remains elusive. High-level synthesis of PspA occurs only under extreme stress conditions including heat shock, cold shock, osmotic shock, and exposure to ethanol (Brissette et al. 1990; Kleerebezem and Tommassen 1993; Model et al. 1997). These stress

conditions might all lead to the dissipation of the proton-motive force, and expression of the PspA may help the cells to maintain the proton-motive force under such stress conditions (Kleerebezem et al. 1996).

The penicillin tolerance protein of *E. sibiricum* 255-15 was also found greatly overexpressed at 4°C. In *P. articus* 273-4, 18 proteins were up-regulated at 4°C in ½ TSB and only four proteins were up-regulated at 4°C in ½ TSB supplemented with 5% NaCl (Zheng et al. 2007). These facts suggest that a single stress could induce other stress-induced proteins that are organized in a complex and highly sophisticated adaptation network.

12.2.4 Cold-Adapted Enzymes

Enzymes which exhibit high catalytic efficiency at low temperatures are called cold-adapted enzymes. Indeed, cold-adapted enzymes have been isolated from cold-adapted organisms including psychrotrophic and psychrophilic bacteria. While cold-active enzymes are characterized by a high catalytic efficiency at a low temperature, they may behave differently at moderate temperatures: some of them exhibit a high catalytic efficiency at moderate temperatures but are rather thermolabile, others inactivate rapidly at a moderate temperature (Feller et al. 1996). However, not all enzymes found in psychroactive organisms are cold-adapted. Many enzymes of psychrophiles show comparable thermostability and catalytic efficiency to the counterparts of mesophilic organisms (Brenchley 1996). In general, rates of biochemical reactions are reduced under low-temperature conditions. However, since levels of the growth rates of psychrophilic bacteria are comparable to those of homologous organisms living at a moderate temperature, relatively similar metabolic rates must be maintained in psychrophilic bacterial cells. For achieving metabolic rate compensation, two enzymatic mechanisms have been proposed: (1) alterations in the concentration of enzymes present in the cells, and (2) changes in the catalytic efficiencies of enzymes (Hochachka and Somero 1984). For instance, an increase of enzyme concentration and activity in the *Lactococcus lactis* has been reported during cold adaptation (Wouters et al. 2000). Overexpression of polynucleotide phosphorylase has been detected in *E. coli* at low temperatures (Mathy et al. 2001).

E. sibiricum 255-15 is able to grow efficiently at temperatures down to -6°C (Vishnivetskaya et al. 2007); therefore, clearly, this organism has found mechanisms of temperature compensation in order to cope with the reduction of chemical reaction rates induced by low temperatures. A proteomics study of cold-adapted cells of *E. sibiricum* 255-15 showed that 28 out of 39 identified CAPs were enzymes. The higher levels of triosephosphate isomerase, acetolactate decarboxylase and cyclohydrolase have been detected in cells of *E. sibiricum* 255-15 grown at low temperature (Qiu et al. 2006). Cold-adapted enzymes in psychrophilic organisms may catalyze rate-limiting steps in metabolism, and play essential roles in survival at a low temperature. Another mechanism for survival is to express

enzymes with temperature-independent reaction rates. This is the case of perfectly evolved enzymes, where such enzymes are relatively rare: typical examples are carbonic anhydrase, acetylcholinesterase, and triosephosphate isomerase. Perfectly evolved enzymes, apparently, do not need to be adapted to low temperatures from a kinetic point of view, therefore they could be extremely useful to probe the various hypotheses related to enzyme adaptation. It may be suggested that the possible role of these enzymes involves maintenance of the bacterial metabolism enabling the cells to adapt to cold temperatures.

12.2.5 *Housekeeping Protein*

Every microorganism contains a set of proteins involved in the basic functioning of a cell. These proteins are called the housekeeping proteins. The synthesis rate of these “common” proteins does not vary significantly with growth temperature. From a 2D-map of *P. cryohalolentis* K5, a total of 311 (51%) of the spots did not vary with growth temperature (-4°C , 4°C and 16°C) and accounted for 73% (v/v) of the amount of protein detected at each temperature (Bakermans et al. 2007). The proteome of *E. sibiricum* 255-15 showed that most of the proteins were similarly expressed at the two temperatures, 4°C and 25°C (Qiu et al. 2006). While housekeeping proteins are required for basic cell functions at any temperature, they may be essential for the proper function of the bacterial cells during the cold-adaptation process.

12.3 Putative Roles of Cold-Inducible Proteins in Low-Temperature Growth

The temperature regulates the growth rate, the level of biosynthesis, metabolism, and survival (Price and Sowers 2004). Comparison of the proteomic profiles of different psychroactive bacteria grown at low temperatures involves the up-regulation of the similar proteins.

Protein profiles of strains *P. cryohalolentis* K5 and *E. sibiricum* 255-15 following cold adaptation showed overexpression of translation elongation factor Ts involved in gene expression, and F1/F0-type ATP-synthase B subunit important for energy production (Qiu et al. 2006; Bakermans et al. 2007). The overexpression of translation elongation factor Tu was observed in two *Psychrobacter* strains studied (Bakermans et al. 2007; Zheng et al. 2007). The proteins involved in gene expression, e.g., CSPs, transcriptional regulators, ribosomal proteins, RNA chaperones and elongation factors, are known to be induced in the response to low temperature in order to decrease stress on transcription, translation initiation and elongation (Mihoub et al. 2003). Low-temperature-induced synthesis and accumulation of CIPs in the cells allows bacteria to maintain energy and constructive metabolism under unfavorable environmental conditions.

Bacteria of the genus *Exiguobacterium* are non-spore-forming bacteria; however, the elevated level of the sporulation control protein was observed in both *Exiguobacterium* strains studied, suggesting that cold-stressed bacteria may enter cyst-like resting states that enhance their survivability (Chong et al. 2000; Qiu et al. 2006; Soina et al. 2004). Growth at low temperatures has been shown to require more energy and be less efficient (Bakermans et al. 2003; Bakermans and Neelson 2004). Both *Exiguobacterium* strains showed low-temperature overexpression of triosephosphate isomerase that involved glycolysis which might be maximally induced under cold growth (Wouters et al. 2000). Some bacteria use different pathways at different growth temperatures; for example, psychrotrophic *Rhizobium* strains switched from respiration to lactate glycolysis in order to generate energy effectively at low temperatures (Sardesai and Babu 2000). Temperature-specific carbon source utilization has also been observed in *E. sibiricum* 255-15 and *P. arcticus* 273-4 (Ponder et al. 2005). Various carbon sources may differentially influence the protein production, suggesting that cells grown with one carbon source may be stressed by low temperatures to a greater extent than cells grown with another (Barbaro et al. 2002). The suggested induction of the glycolysis at low temperature has been further supported by observation of up-regulation of the enzymes of the glycolytic pathway, e.g. malate/lactate dehydrogenases, in *P. cryohalolentis* K5 (Bakermans et al. 2007).

The affinity to substrate decreases at low temperatures; therefore the changes in transport systems are required to counteract lower rates of diffusion and solute transport across the membrane (Nedwell 1999). Bacteria of the genera *Exiguobacterium* and *Psychrobacter* were shown to be able to grow at temperatures below 0°C, therefore the processes of substrate sequestration from the environment and excretion of spent solutes from cells turn out to be very important for growth at the low temperatures. A number of transport-related proteins and membrane-associated proteins were up-regulated by cold in these strains (Chong et al. 2000; Qiu et al. 2006; Bakermans et al. 2007; Zheng et al. 2007). The drop of a temperature below 0°C leads to ice formation within the cell which might lead to cell lysis, and leads to the increase of salinity outside the cell followed by the consequent increase of an osmotic gradient across the cell membrane. The cold-shock induced ice nucleation activity in different psychroactive bacteria including *E. sibiricum* 7-3 (Ponder et al. 2005), and induced synthesis of the ice nucleation proteins which can act as a template for ice formation (Kawahara 2002). Another stress that bacteria encounter at low temperatures is oxidative stress, because oxygen radicals accumulate to higher concentrations, given that oxygen is more soluble and reduced respiration rates consume oxygen more slowly. The CIPs of diverse functions including chemotaxis, hydroperoxide detoxification, and surface proteins may maintain cell integrity and functioning during this stress (Bakermans et al. 2007).

The psychrotrophic bacteria harbored antibiotic multiresistant traits, and this feature increased with cold (Munsch-Alatossava and Alatossava 2007). While *E. sibiricum* 255-15 showed a decrease in resistance to chloramphenicol and tetracycline at 4°C (penicillin was not tested) (Ponder et al. 2005), the high overexpression level of penicillin tolerance protein was detected in this bacterium at 4°C (Qiu et al. 2006).

During the growth at low temperatures, cells cope with amino acid starvation, oxidative stress, aberrant protein synthesis, cell-surface remodeling, alterations in degradative metabolism, and induction of global regulatory responses. A life in less than ideal environmental conditions leads to changes in the physiological state and the biochemical activity of bacterial cells, and these changes bind directly to protein synthesis.

12.4 Putative Roles of Differentially Induced Proteins in Cryotolerance

There has been growing interest in the survival mechanisms of psychrotolerant bacteria at repeated freeze–thaw cycles largely because successive freezing and thawing are common processes in nature. In addition, there is a considerable interest in the cryotolerance mechanisms of both bacteria related to food-spoilage and food-borne pathogens. It appears that overexpression of CSPs significantly improves cryotolerance, and helps to retard freezing or lessen the damage incurred upon freezing and thawing of the bacteria, yeasts, and plants (Kim et al. 1998a; Thomashow 1998; Broadbent and Lin 1999; Wouters et al. 1999; Thammavongs et al. 2000; Wouters et al. 2001; Minami et al. 2005).

In order to characterize freeze–thaw resistance, the single-cell isolates of the genus *Exiguobacterium* were subjected to repetitive freeze–thaw cycles (Vishnivetskaya et al. 2007). This study showed that bacteria grown in complex, structured (agar) medium had improved tolerance to the freeze–thaw challenge compared to bacteria grown in mass-action (liquid) medium, regardless of growth temperature. However, growth temperature was a determining factor of a cryotolerance in mass-action (liquid) habitat. Bacteria grown at 4°C in liquid medium tolerate freezing/thawing much better than when grown at 25°C. A subsequent study compared proteomic profiles of *E. sibiricum* 255-15 grown in liquid broth or an agar surface at both 4°C and 25°C to determine proteins important for cryotolerance (Qiu et al., unpublished). The bacteria with improved cryotolerance have revealed a general down-regulation of enzymes involved in major metabolic processes (glycolysis, anaerobic respiration, ATP synthesis, fermentation, electron transport, and sugar metabolism) as well as in the metabolism of lipids, amino acids, nucleotides and nucleic acids, while eight proteins (2′–5′ RNA ligase, hypoxanthine phosphoribosyl transferase, FeS assembly ATPase SufC, thioredoxin reductase and four hypothetical proteins) were up-regulated (Qiu et al., unpublished). It has been shown that the repression of RNA species and over-expression of enzymes involved in amino acid biosynthesis during nutritional deprivation led to improved bacterial survivability (Jain et al. 2006). The overproduction of the CSPs in the mesophilic bacterium *Lactobacillus plantarum* transiently alleviated the reduction in growth rate, and led to an enhanced capacity to survive freezing (Derzelle et al. 2003). In *E. sibiricum* 255-15, only 15% of the total cellular proteins were overexpressed more than two-fold under different growth conditions. The induction of these proteins might have a potential role in freeze–thaw resistance.

The suppression of some enzymes in the cells grown on agar or at low temperatures indicated the reduction of biochemical reaction rates at these conditions. Therefore, it is reasonable to assume that bacterial cells with slowed metabolism and an enhanced system of replication, recombination, and repair easily tolerate severe environmental factors, e.g., repetitive freeze–thaw cycles.

12.5 Conclusion

The studies described in this chapter indicate that the adaptive nature of permafrost bacteria at near-freezing temperatures is regulated by cellular physiological processes through the regulation of certain cellular proteins. Although cold adaptation is still far from being properly understood, it is possible that proteins synthesized at low temperatures may support temperature homeostasis, protect other proteins from denaturation and damage, and enable the cells to adapt to near or below-freezing temperatures.

Acknowledgements This work was supported by National Aeronautics and Space Administration (NASA) Astrobiology Institute under cooperative agreement no. CAN-00-OSS-01 issued through the Office of Space Science.

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