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MINIREVIEW

Coping with Our Cold Planet[▽]

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Of all the natural stress conditions on our planet and in our solar system, cold is arguably the most widespread, at least from the perspective of mesophilic and thermophilic organisms. For instance, 90% of the Earth's oceans have a temperature of 5°C or less. When terrestrial habitats are included, over 80% of the Earth's biosphere is permanently cold (149). Among terrestrial environments, 85% of Alaska, 55% of Russia and Canada, 20% of China, and the majority of Antarctica are permanently cold (136). Furthermore, six of the other eight planets of our solar system are permanently cold, and hence understanding life's adaptations to cold environments on our planet should be useful in the search for and understanding of life on other planets. Indeed, the current debate about whether life exists in Lake Vostok or on other planets is rooted in understanding what we know about microbial life in cold environments (83; C. Zimmer, *New York Times*, 6 July 2007).

Since water is the major constituent of life, the manner in which its physical properties depend on temperature is fundamental to understanding the mechanisms that govern cold tolerance and acclimation. A number of Earth's microorganisms have the ability to cope with low temperatures, which makes them among Earth's most successful colonizers (62, 63). In this review we summarize how physical properties of water at cold temperatures affect the physiology of microorganisms and we focus on the molecular mechanisms revealed by recent biochemical and genetic studies that shed light on microbial adaptations to cold.

Effects of cold on physical properties of water and on biochemical reactions. Properties of water have a profound influence on the physical and chemical processes that are essential for life (51). Water is the only chemical compound that occurs naturally in solid, liquid, and vapor phases and the only naturally occurring inorganic liquid on Earth (51). The water molecule consists of two hydrogen atoms and one oxygen atom. The HOH angle is 104.5°, and the intramolecular OH distance is 0.957 Å (37). A strong, directional attraction occurs between an oxygen atom and a hydrogen atom belonging to a different water molecule, i.e., a hydrogen bond (168). At temperatures below 4°C, hydrogen bonds force energy and volume, as well as entropy and volume, to be negatively correlated, while the correlations are positive for most liquids. The negative correlation between entropy and volume is a consequence of the formation of an open hydrogen-bound network in which a decrease in orientation entropy is accompanied by an increase in volume (37). This tendency of water molecules to attract each other strongly through hydrogen bonds is responsible for the unusual properties of water (37).

In a subzero environment, water can be supercooled or frozen (52). Supercooling is not accompanied by changes in the concentrations of water-soluble substances, except in rare cases where such substances might be present at near-saturation concentrations at normal temperatures and precipitate under supercooling conditions. Supercooling conditions in aqueous systems can persist at temperatures down to −40°C and at even lower temperatures (37, 52, 147). At low and subzero temperatures the physical properties of liquid water change substantially. The changes are not linear but become more pronounced at lower temperatures. Some of the changes at lower temperatures involve expansion of the water molecule, increases in viscosity, dielectric permittivity, compressibility, and pK_w (the negative log of the water ion product), and decreases in diffusion (2, 37). However, the most dramatic effect, at least from the viewpoint of biochemistry, is the degree of ionization (78). As H^+ and OH^- ions are involved in most reactions in living organisms (condensation, hydrolysis, oxidation, and reduction), a decrease in K_w affects equilibrium and kinetic processes (52).

Besides being supercooled, water can also be in a frozen state. In the crystalline state, water has 13 known polymorphs, nine of which (ices II, III, V, VI, VII, VIII, X, and XI and ordinary hexagonal ice, Ih) are stable over a certain range of temperatures and pressures and 4 of which are metastable (ices IV, IX, and XII and cubic ice, Ic) (108, 116, 133). Water, like any other liquid, can also be vitrified when it is cooled sufficiently fast to avoid crystallization. At least two distinct forms of vitrified water are found: low-density and high-density amorphous ice. Low-density amorphous ice can be formed when water vapor at low pressure is deposited on a sufficiently cold substrate. High-density amorphous ice is formed by application of pressure to ice Ih at a low temperature (37). We are not aware of studies that show which kinds of ices can be formed intracellularly at different cooling rates, temperatures, and pressures, even though the cooling velocity is one of the major factors that determine whether a cell is viable after freezing. Cooling either too slowly or too rapidly can be damaging (122). If cooling is sufficiently slow and ice forms extracellularly, the cell loses water rapidly by exosmosis, resulting in

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increased concentrations of the intracellular solutes that maintain the chemical potential of intracellular water in equilibrium with that of extracellular water. The result is that the cell dehydrates and does not freeze intracellularly. But if the cell is cooled too rapidly, it is not able to lose water fast enough to maintain equilibrium and can eventually freeze intracellularly. Ideally, for better cell survival at subzero temperatures, cells should be cooled slowly enough to avoid intracellular ice formation by increasing the solute concentration intracellularly and to permit the cells to physiologically adapt to the new temperature conditions (122).

Besides the fact that changes in the properties of water can affect cell integrity, water is also essential for most biochemical reactions. In virtually all natural environments, energy or sometimes substrates necessary for growth (phosphates, sulfates, and nitrogenous compounds, among others) are present at rate-limiting levels (128). Growth and survival depend, therefore, on the ability of organisms to sequester these scarce resources more successfully than competing species. Reductions in the substrate diffusion rates and the increase in the viscosity of the water in the environment of a cell at cold temperatures make it more difficult for the cell to acquire resources. The efficiency of active uptake at low concentrations depends on the substrate affinity of the uptake mechanisms (128). The affinity for substrate uptake is frequently described by Monod-type saturation curves relating the growth rate to the concentration of the rate-limiting substrate (71). The general paradigm is that when the environmental temperature decreases below the optimum temperature for growth, there is a concomitant decrease in the affinity for substrates, as measured by maximum specific growth rate/K_s. The trend toward decreased affinity at lower temperatures applies to the uptake of both organic and inorganic substrates (79, 129, 140).

A common strategy used to sustain cell activity at a permanently low temperature is to produce cold-adapted enzymes with enhanced catalytic efficiency (48, 57). In general, effective substrate uptake and catalysis by enzymes at low temperatures require increased specific activity (k_{cat}) (34, 35, 46, 134, 159). The high reaction rates of several cold-adapted enzymes (up to 10-fold higher than the reaction rates of heat-stable homologs) are due to decreases in the activation of the free energy barrier between the ground state (substrate) and the transition state (159). Studies with GTPases (160), chitanase (118), isocitrate dehydrogenase (177), and xylanase (1) suggested that the k_{cat} could be increased by an indirect increase in the entropy of the system via water dislocation (159, 180). It has been suggested that if more water molecules are released upon binding of the transition state to the enzyme than upon binding of the ground state, there will be considerable entropic benefit if an enzyme transition state complex is formed (115, 117, 164, 180). Alternatively, for secreted enzymes in marine environments or intracellular enzymes that face low substrate concentrations, a decrease in K_m provides higher substrate affinity for many psychrophilic enzymes (34, 35, 50, 159). For instance, the K_m values for pyruvate differ by a factor of 3 for the hyperthermophilic and psychrophilic lactate dehydrogenases (34). The strength of the ground state enzyme-substrate interaction may increase (electrostatic interactions) or decrease (hydrophobic interactions) with decreasing temperature (159). Both types of interactions are affected by changes in water structure that occur as a function of temperature (107). For instance, the overall free energy change that occurs during the formation of an ionic bond is complicated by the effect of temperature on the dielectric constant of water (159). Increased enzymatic structural flexibility, however, allows better enzyme interaction with substrates and can reduce the activation energy necessary to generate reaction intermediates, resulting in more efficient substrate turnover (45, 57, 132). The flexibility/rigidity concept refers to conformational changes in the milli- to microsecond time range that occur during catalysis and not to the very fast atomic fluctuations (picosecond) of protein structures (184). The very fast dynamic motions, which are thought to have the primary role in protein flexibility, are connected to the dynamics of surrounding water molecules (47, 77, 163, 169).

Extensive studies of the structure of psycrophilic enzymes have demonstrated that the flexibility of these enzymes is the result of a combination of several features, including an increase in the number of hydrophobic side chains that are exposed to the solvent, a decrease in the compactness of the hydrophobic core, a higher number of glycine and lysine residues, a reduced number of proline and arginine residues, and weakening of intramolecular bonds (fewer hydrogen bonds and salt bridges compared to mesophilic and thermophilic homologs) (33, 35, 57, 59, 81, 151, 162). All these features are not present in the same cold-active enzymes, but these features are some of the characteristic changes found in cold-active enzymes compared to their mesophilic and thermophilic counterparts (151).

The hydrophobic interaction is the basis of the process of folding and the stabilization of protein associations (32, 92). The hydrophobic interaction occurs when apolar residues aggregate in an aqueous environment, resulting in a loss of free energy that stabilizes the protein structure. During association of monomers, hydrophobic residues are concentrated in the interfacial regions, minimizing the number of thermodynamically unfavorable solute-solvent interactions (170). Inside the core of a cold-adapted enzyme, buried residues show weaker hydrophobicity than residues in its mesophilic homologs, making the protein interior less compact and more flexible (102, 152).

Additionally, glycine residues have also been suggested to contribute to cold adaptation in a protein (45, 111, 121) by increasing the thermal agitation. A site-directed mutagenesis study of cold-adapted lactate dehydrogenase demonstrated the role of Gly (49), since the wild-type enzyme had lower free activation energy than the mutants. Other amino acids, however, such as arginine, have a higher hydrogen bonding potential. As a consequence, arginine occurs less frequently in psychrophilic enzymes than in mesophilic enzymes (58, 59, 112, 162).

Furthermore, the possible contribution of electrostatics to active site flexibility and proper solvation of psychrophilic proteins has only recently been considered. For instance, citrate synthase (106) and elastase (131) are psychrophilic enzymes that have higher proportions of charged residues and fewer salt bridges than their mesophilic counterparts. The overall increased occurrence of charged residues in a psychrophilic enzyme ensures proper solvation at low temperatures. The salt bridges and their networks are also weaker and more dispersed in a psychrophilic enzyme, and the charged active site residues

TABLE 1. Summary of proteins implicated in adaptation to cold stress

Protein(s)	Function(s)	Reference(s)
AceE	Decarboxylation of pyruvate (pyruvate dehydrogenase)	87, 91
AceF	Dihydrolipoyltransacetylase (pyruvate dehydrogenase)	87
CspA	RNA chaperone	68, 138, 182
CspB	RNA/DNA chaperone (?)	110, 138
CspE	Regulation of CspA	5
CspG	RNA/DNA chaperone (?)	127, 138
CspI	Unknown	176
CsdA	RNA unwinding activity	90, 171
DnaA	DNA binding and replication (initiation); transcriptional regulator	3
RbfA	30S ribosomal binding factor	88
InfA	Initiation factors; binding of charged tRNA-fmet to the 30S ribosomal subunit	91
InfB		91
PNP	Degradation of RNA	9, 91, 120, 183
Hsc66	Molecular chaperone	113
HscB	DnaJ homolog	113
HU-β	Nucleoid protein; DNA supercoiling	60, 125
Trigger factor	Prolyl-isomerase activity and other functions	98
RecA	Recombination factor	91
GyrA	DNA topoisomerase	72, 89, 119
H-NS	Nucleoid-associated DNA-binding protein	42, 109, 138
NusA	Involved in termination and antitermination	91
OtsA	Trehalose phosphate synthase	97
OtsB	Trehalose phosphatase	97
Desaturases	Unsaturation of membrane lipids	72, 109, 126
Dihydrolipoamide acetyltransferase	Decarboxylation of pyruvate	91
Alpha-glutamyltranspeptidase	Glutathione metabolism	144

are electrostatically more destabilizing, which may lead to greater conformational flexibility (131). An early calculation by Honig and Hubbell estimated that the cost of transferring salt bridges from water to the protein environment is approximately 10 to 16 kcal mol^{-1} (80). This large desolvation penalty, due to the burial of polar and charged groups in the protein interior during folding, is generally not recovered by favorable interactions in the folded state. In general, the hydration free energy of amino acids changes with temperature, which is due to an increase in viscosity and surface tension, a decrease in the dielectric constant of water, and the change in the contribution from entropic effects. The effects of temperature on protein hydration and estimates of the free energies of folding proteins indicate that there is an increased desolvation penalty for salt bridges at low temperatures (105). This desolvation penalty explains the observed reduction of salt bridges in psychrophilic enzymes and, once more, shows the importance of water in enzymatic reactions.

The valuable role of water in cell survival and cell reactions is unquestionable. Nonetheless, studies integrating understanding the role of cold-adapted proteins in cell survival with the physicochemical properties of cold water are still in their infancy. Future research in these areas should boost our understanding of how cold adaptation mechanisms are translated into physiology and hence the viability of life forms in extreme environments.

Microbial responses to cold. Cold is a physical stress that drastically modifies all physical and chemical parameters of a living cell and thereby influences solute diffusion rates, enzyme kinetics, membrane fluidity and conformation, flexibility, topology, and interactions of macromolecules, such as DNA, RNA and proteins. In order to overcome the physicochemical effects caused by cold stress, a number of cellular modifications may occur (Table 1). DNA becomes more negatively supercoiled, and nucleoid-associated proteins, such as gyrase A (*gyrA*) and DNA-binding proteins HU-β (*hupB*) and H-NS (*hns*), may be necessary for its relaxation (74, 153, 178). Studies with *Escherichia coli*, however, have demonstrated that *hns* is essential for extended growth in the cold but not for cell survival immediately after cold shock. Additionally, *hns* null mutants display severe morphological alterations and eventually lyse in the cold (42). A cold shock study performed with *Shewanella oneidensis* MR-1 also detected high levels of expression of three genes encoding DNA-binding proteins in the HU family with high levels of similarity to *hupA* and *hupB* in *E. coli*. Among other DNA-modulating proteins in *S. oneidensis*, DNA topoisomerase III was induced, as opposed to *gyrA* in *E. coli* (56). Hence, several genes seem to be involved in the maintenance of functional DNA topology for cell survival at cold temperatures.

The transcription system is also affected by the cold. For example, the transcription factor NusA is known to be involved in both termination and antitermination of transcripts in *E. coli*, and it is highly expressed at lower temperatures (53, 144). NusA is also highly expressed in *S. oneidensis* MR-1, as determined by a global transcriptome analysis of the cold shock response; however, its function in the *S. oneidensis* cold shock response is unknown (56). Besides transcription and translation of mRNA, the cells need to cope with all the transcripts produced. DEAD RNA helicases (encoded by *cdsA* in *E. coli* and *cshA* and *cshB* in *Bacillus subtilis*) have been implicated in the stabilization and degradation of mRNAs (4, 82). In *E. coli*, the helicases accumulate during the early stages of cold acclimation and assemble into degradosomes with RNase E synthesized in cold-adapted cultures (141). CsdA may be involved in the efficient and selective degradation of Csp (cold shock)

mRNAs by unwinding the mRNA secondary structure that impedes the processive activity of 3′-5′ exonuclease polynucleotide phosphorylase (4, 9, 183, 185). Defects in mRNA degradation produce a cold-lethal phenotype displayed by *pnp* mutants of *Yersinia enterocolitica* (130) and *E. coli* (183). A *csdA* gene deletion mutant of *E. coli* has a reduced growth rate and exhibits filamentous growth at low temperatures (25, 74, 90). A mutation study of the first two amino acids of the DEAD box of CsdA showed the importance of these amino acids for the cold acclimation activity of CsdA (4). The same study also showed that a lack of *csdA* function can be complemented by either two cold shock-inducible proteins (CspA, an RNA chaperone, and RNase R, an exonuclease) or another DEAD box RNA helicase, RhlE (4). The absence of CsdA and RNase R, however, results in increased sensitivity of the cells to moderate temperature downshifts. Besides the important role of CsdA in mRNA decay (101, 183), CsdA has also been identified as a multifunctional protein essential at low temperatures. Recently, it was shown that CsdA is involved in the biogenesis of the 50S ribosomal subunits. Deletion of the *csdA* gene leads to a deficiency in free 50S subunits and accumulation of a 40S-like particle (25). The authors showed that CsdA associates with 50S precursors at low temperatures and can complement the ribosome defect of an *srmB* deletion strain (25). In *B. subtilis*, single-deletion mutants with mutations in the *cshA* or *cshB* gene do not exhibit any growth defects after cold shock; however, double deletion of the helicases is lethal for *B. subtilis* (82). Additionally, combined deletion of the helicase gene *cshB* and the major cold shock protein (CSP) gene *cspB* in *B. subtilis* results in slow growth after a cold shock, revealing that *cshB* and *cspD* have a strong synergistic effect (82). Together, these data suggest that the DEAD box RNA helicases are essential for CSPs in different organisms. Even though in *E. coli* CsdA has multiple overlapping functions in several important physiological processes, such as processing or degradation of RNA or ribosome biogenesis (84), in other organisms these functions have not been proven yet.

For translation, ribosome assembly appears to be affected by cold stress, as suggested by the cold-sensitive phenotype displayed by many *E. coli* and yeast ribosome assembly mutants (38). In light of these findings, it is likely that when this process is interrupted by a sudden cold stress or when de novo ribosome assembly is started at a suboptimal temperature, resumption requires the presence of new types and/or additional copies of proteins, including RNA chaperones and helicases. Among the genes encoding ribosome-associated proteins in *E. coli* which are translated during cold stress are the genes encoding RNase R, the cold shock protein A (*cspA*) family, DEAD box RNA helicase (*csdA*), initiation factors (IF), and ribosome-binding factor A (*rbfA*). In *S. oneidensis* MR-1, translational IF1 and IF2 (*infA* and *infB*) and *rbfA* were also induced upon cold shock (56). In a recent study (22), it was shown that in *E. coli* the level of the RNase R protein was increased seven- to eightfold upon cold shock at 10°C. RNase R is a 3′to 5′ exoribonuclease (29, 31) and plays a major role in the degradation of repetitive extragenic palindromic sequences in mRNA (28) and other secondary RNA structures, such as rRNA, for quality control purposes (30), and defective tRNA (114). Chen and Deutscher suggested that during cold shock, a significant portion of ribosomes are misassembled and RNase R could contribute to their turnover, akin to the known role of this enzyme in rRNA quality control (27). In *B. subtilis*, the ribosome rescue system was described as consisting of the SsrA and SmpB proteins. Loss of the function of these proteins has a significant effect on growth at both high and low temperatures in *B. subtilis* (155). The former protein functions as both a tRNA and an mRNA to rescue ribosomes that are unproductively stalled on an mRNA (100, 161); the latter is a small ribosome-binding protein essential for transfer-messenger RNA (*ssrA*) function (155). Among the CspA family proteins in *E. coli* (CspA through CspI), only CspA, CspB, CspG, and CspI are cold shock inducible; CspC and CspE are constitutively produced at 37°C, and CspD is induced by nutritional deprivation and in the stationary phase at 37°C (44, 137, 178). *E. coli* cells having single, double, or triple deletions of cold-inducible *csp* genes grow at low temperatures, suggesting that none of the CspA homologues alone is responsible for cold shock adaptation (181). CspE is induced by cold shock in a triple deletion mutant (Δ*cspA* Δ*cspB* Δ*cspG*), while a quadruple deletion strain (Δ*cspA* Δ*cspB* Δ*cspG* Δ*cspE*) is cold sensitive, suggesting that the cold acclimation function(s) of CSPs is redundant and that CspE, which is not induced normally by a cold shock, can perform essential cold acclimation functions. In fact, the quadruple deletion mutant can be complemented during growth at a low temperature by overproduction of any one of the nine CSP family genes (except *cspD*) (181). CspA, CspC, and CspE bind RNA and single-stranded DNA with low affinity and specificity, acting like a chaperone (86, 139). The binding leads to energy-dependent melting and/or destabilization of nucleic acid secondary structure elements that favors transcription and translation under cold stress conditions (6, 86, 139, 142). Whereas *E. coli* has nine CSPs in its genome, *S. oneidensis* contains three CSPs and only one of them, which has high sequence similarity to *E. coli* CspA, is highly expressed during cold shock (56). *Vibrio cholerae*, on the other hand, has genes encoding four CSPs in its genome (76), and *B. subtilis* contains three strongly cold-induced CSPs (CspB, CspC, and CspD) (21, 82, 178). Additional help to unwind the RNA secondary structure and to facilitate translation at low temperatures is also provided by the gene product of the DEAD box RNA helicase, as discussed above (43, 144). The initiation process in protein synthesis, on the other hand, requires three key protein factors in *E. coli* (IF1, IF2, and IF3). After cold stress, the levels of the IFs increase due to the cold shock translational bias, which is one of the main and most characteristic mechanisms ensuring that a cell selectively expresses its cold shock genes (65–67, 103). The bias is due to *cis* elements in the cold shock mRNAs that make the mRNAs more prone to translation at low temperatures and to *trans* elements associated with the translational apparatus of the cold-shocked cells. After a cold shock, *infA* and *infC*, which code for IF1 and IF2, respectively, are transcribed de novo primarily from two of their promoters that are used less at 37°C. These promoters that are less used at 37°C are also more distal from the two genes, which yields mRNAs with a longer 5′ untranslated region that has been suggested to increase the stability of the transcripts and/or to favor their translation in the cold (65, 103). After translation of the proteins, correct protein folding in the cold could be problematic and require the presence of chaperones, such as the DnaK homologue

Hsc66 (113) and perhaps IF2 (23), while direct or indirect participation of the trigger factor in this process has also been suggested (74).

Besides the production of cold-induced proteins, several other cold stress cellular responses maintain cell viability. It is well known that cells synthesize unsaturated and branched fatty acids so that membranes do not become less fluid at cold temperatures (148, 150). This phenomenon has been studied in detail in two cyanobacteria, *Anabaena variabilis* and *Synechocystis* sp. strain PCC 6803; mutants of these bacteria defective in the desaturation of fatty acids (*desA*) have a lower growth rate at low temperatures (175). Grau and collaborators (72) proposed that DNA supercoiling may regulate unsaturated fatty acid synthesis in *B. subtilis* since the desaturation of fatty acids induced after transfer to a low temperature is inhibited by the addition of novobiocin, an antibiotic that affects DNA gyrase activity. More recently, genome analyses of the psychrophile *Colwellia psychrerythraea* 34H revealed the presence of a suite of coding sequences encoding polyunsaturated fatty acid synthesis that maintain the cell membrane liquid-crystalline state (124).

Although increased unsaturation and decreased chain length of fatty acids are the major modifications of cell membranes, other membrane-associated molecules may also play important roles in adaptation to low temperatures (26, 85, 144). For example, in vitro studies of Antarctic psychrotrophic bacteria have indicated that carotenoid pigments interact with the cell membrane and increase its rigidity (85). Since a large number of Antarctic bacteria contain carotenoid pigments in the cell membrane, it has been postulated (85) that these pigments may have a role in buffering membrane fluidity when there is an increase in the environmental temperature, thereby maintaining the homeoviscosity of the membrane prior to the de novo synthesis of saturated fatty acids.

Further analyses of the genome sequence of *C. psychrerythraea* 34H also revealed the capacity to produce polyhydroxyalkanoate compounds, a family of polyesters that serve as intracellular carbon and energy reserves, as well as the capacity to synthesize and degrade polyamides similar to cyanophycin that function as nitrogen reserves (124). The collective gene complement for biosynthesis of polyhydroxyalkanoate and cyanophycin-like compounds was suggested to ensure intracellular reserves of nitrogen and carbon to aid in circumventing any cold-imposed limitations to carbon and nitrogen uptake (124). The presence of intracellular granules in cold-adapted microorganisms has also been described in a psychrotroph isolated from the Siberian permafrost, *Exiguobacterium sibiricum* 255-15 (145). Additional genome analysis of *C. psychrerythraea* also showed the ability of this cold-adapted microorganism to take up or produce compatible solutes, such as glycine betaine and extracellular polysaccharide compounds that can serve as cryoprotectants (124).

Cells likely undergo a number of modifications in order to survive and grow at extreme temperatures. Networks of genes, which are activated simultaneously or in cascade fashion, likely generate these modifications. How the cascade of genes is activated and the phenotypic effects of the genes in different microorganisms have not been explored, especially in the psychrotrophs and psychrophilic microorganisms that are prevalent in naturally cold environments. The regulatory networks involved in cold sensing and response are likely to be a fruitful topic for future research.

What microbes live in cold habitats. The lowest temperature reported for microbial activity is −20°C in permafrost soil and sea ice (36). Cold-adapted microorganisms have been found in Antarctic subglacial soil and lakes, cloud droplets, ice cap cores obtained from considerable depths, snow, glaciers, and cold deep-sea environments (41, 167). Even though cold-adapted microorganisms can be found in diverse environments, culture and culture-independent diversity studies of cold-adapted microbes have mostly been done in deep-sea waters, sea ice, and permafrost environments (20, 24, 55, 99, 174). In the cold interior and deep waters of the ocean, well below the upper mixed layer, *Archaea* occur at densities greater than the densities of their counterparts in surface waters (10, 41, 93, 143) and greater than the densities of *Bacteria* at the same depths (99). In the deep Pacific Ocean, populations of *Crenarchaeota* largely account for the archaeal presence, and the population densities match or exceed the bacterial densities (99).

The total microbial counts in Arctic pack ice and in Antarctic sea ice were determined to be 10^5 and 10^6 cells ml^{-1}, respectively (19). Cultivation approaches, mainly used with Antarctic samples, provided the initial view of the diversity of sea ice bacteria and revealed several novel genera and species (13–17, 69, 70, 123). Most of the isolates were psychrotolerant and grew optimally at temperatures between 20 and 25°C. Only a few strains were psychrophilic and grew optimally at 10 to 15°C. Among the sea ice prokaryotes, members of the following eight phylogenetic groups have been found: the α, β, and γ subclasses of *Proteobacteria*, the genera *Cytophaga*, *Flavobacterium*, and *Bacteroides* in the *Bacteroidetes*, the *Actinobacteria* (high G+C contents), and the *Firmicutes* (low G+C contents) (19, 20, 73, 135). rRNAs from archaea associated with sea ice comprised as much as 34% of the total prokaryotic rRNA obtained from some samples, and these rRNAs were from members of both the *Euryarchaeota* and the *Crenarchaeota* (39, 40). Whereas culture-independent methods have revealed the same phylogenetic groups in sea ice from both poles, cultivation-based studies have provided a rather disjunctive picture of culturable sea ice bacteria (19, 20, 94, 165), probably due to the different cultivation methodologies.

Viruses have also been detected in Arctic sea ice, but this domain is the least explored domain in cold habitats. A marine virus-host system that infects psychrophilic bacteria has been demonstrated for Arctic sea ice, and this system forms plaques at temperatures as low as 0°C (11, 12, 172, 179). While it might be expected that host-virus systems would also be important to the ecology and evolution of microbes in cold environments, it is not known how their adaptations and their relative roles in nutrient dynamics and in host evolution vary compared to the adaptations and roles of their mesophilic counterparts.

Additionally, the diversity of microorganisms in the interface between the solid and liquid phases of sea ice (the eutectic interface) has not been explored. This environment is the coldest ice habitat (−20°C) examined so far for microbial life. In situ microscopic studies have shown that living bacteria (eutectophiles) can be found in this interface (95). Kanavarioti and collaborators (96) found that the ice matrix, with its concentrated solutes in the spaces between ice crystals, can favor oligonucleotide polymerization reactions. These findings sug-

gest that microorganisms may be metabolically active and perhaps reproduce in the eutectic interfaces.

Despite the widespread distribution of permafrost, most microbial information on this habitat is information for only a few study sites that cannot encompass the diversity and history of permafrost. The research on northeast Siberian permafrost soils and sediments has been the most extensive and has revealed that the total concentration of bacterial cells ranges from 10^7 to 10^9 cells/g (dry weight) independent of the age of the permafrost (173, 174). Antarctic permafrost, which has a much lower organic carbon content than the Arctic permafrost, contains 10^3 to 10^4 cells/g (dry weight), as determined by epifluorescence microscopy (64). Most isolates from Arctic and Antarctic permafrost are psychrotrophic rather than psychrophilic, although mesophiles and even a limited number of thermophiles have been detected (167). The growing library of the Arctic permafrost isolates includes organisms belonging to nine bacterial classes and more than 35 genera (61, 75, 104, 145, 146, 154, 166, 173, 174). The most common genera include *Arthrobacter*, *Aeromonas*, *Bacillus*, *Cellulomonas*, *Exiguobacterium*, *Flavobacterium*, *Micrococcus*, *Myxococcus*, *Nitrobacter*, *Pseudomonas*, *Psychrobacter*, *Rhodococcus*, and *Streptomyces*. Studies of methanogenic communities in Arctic soils also revealed diverse methanogenic *Archaea*, including members of the *Methanomicrobiaceae*, *Methanosarcinaceae*, and *Methanosaetaceae* (55). In Antarctic permafrost the genera *Cytophaga*, *Bacteroidetes*, *Arthrobacter*, *Bacillus*, *Roseobacter*, and *Streptomyces* were predominant (158, 167), although the genera *Exiguobacterium* (54) and *Psychrobacter* (18, 156, 157), among others, were also found.

More recently, other permafrost environments have been explored, including high Arctic permafrost soil from Spitsbergen (75). Nine bacterial taxa, *Thermomicrobia*, *Mollicutes*, *Spirochaetes*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and three candidate divisions, were detected in 16S rRNA clone libraries. *Actinobacteria* were dominant culturable bacteria in the same Spitsbergen samples, which is consistent with the findings of other investigators who studied Arctic permafrost (8, 104, 174). Another Arctic permafrost studied, which had a relatively high carbon content and was at a more northern latitude than the Siberian permafrost site, is the Eureka permafrost, located in the Canadian high Arctic (166). This study showed that the bacterial 16S rRNA gene library contained rRNAs of *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Planctomyces*, and *Gemmatimonadetes*; the dominant phylotypes were *Actinobacteria* and *Proteobacteria*. The culturable isolates belonged to the *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, and members of the *Firmicutes* were the most abundant organisms. The *Archaea* 16S rRNA gene clone library contained sequences from members of both the *Euryarchaeota* and the *Crenarchaeota*. The majority of the sequences were related to halophilic *Archaea*. Lastly, two studies of alpine permafrost have been performed, one in the Tianshan Mountains in China and the other in the Qinghai-Tibet plateau (7, 186). The total concentrations of bacterial cells obtained in these two studies were 10^5 and 10^7 CFU/g (dry weight), respectively. In these culture-dependent studies, four major groups were found: low- and high-G+C-content gram-positive bacteria, *Proteobacteria*, and *Bacteroidetes*. The most abundant and diverse isolates in both studies were gram-positive bacteria, particularly *Arthrobacter*, which had results similar to the Spitsbergen results (7, 75).

These findings provide some insight into microbial communities in some cold habitats on our planet; however, we still lack information on which members of the communities are viable and active in situ. Several studies have detected novel, uncultivated phylotypes. Isolation and subsequent physiological and genomic characterization of these phylotypes should improve our understanding of the adaptations and role of cold-loving microbes. Progress, however, is dependent on better methodologies to measure activities and to cultivate these microbes. Furthermore, too few microbe-focused studies have been done in other cold habitats, such as alpine permafrost soils, eutectic interfaces, ice-covered lakes, and snow, to provide comprehensive knowledge of life in the cold.

CONCLUSIONS

Understanding the ecology of and adaptations to cold habitats requires not only knowledge of the extant microbial community and the environmental factors that affect survival but also the physiological adaptations to these factors. Obviously, in any environment, water plays an important role in cell survival. In the case of cold environments, water plays an even greater role, since changes in its physicochemical properties at low temperatures directly affect biochemical reactions, including nutrient uptake by microbes and hence the organisms' competitiveness. Microorganisms have coped well with the changes in water properties probably due to their long history on a cold planet, especially by having evolved (i) structurally different proteins with improved catalytic efficiency, (ii) improved mechanisms to guide macromolecules through their needed functions, and (iii) adjustments in the cell envelope to perform its critical functions. Many communities of cold-adapted microorganisms and their physiologies and ecologies have not yet been studied much; however, these microorganisms occupy habitats with probably the most extensive ecological condition (cold) for microbial life on Earth and thus are major sustainers of essential biogeochemical cycles.

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