

Psychrophiles and polar regions

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Most reviews of microbial life in cold environments begin with a lament of how little is known about the psychrophilic (cold-loving) inhabitants or their specific adaptations to the cold. This situation is changing, as research becomes better focused by new molecular genetic (and other) approaches, by awareness of accelerated environmental change in polar regions, and by strong interest in the habitability of frozen environments elsewhere in the solar system. This review highlights recent discoveries in molecular adaptation, biodiversity and microbial dynamics in the cold, along with the concept of eutectophiles, organisms living at the critical interface inherent to the phase change of water to ice.

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Abbreviations

DOM dissolved organic matter
POM particulate organic matter

Introduction

Cold-adapted microorganisms are generally understood to achieve their physiological and ecological successes in cold environments as a result of unique features in their proteins and membranes and their genetic responses to thermal shifts. Relative to mesophilic counterparts, a cold-active enzyme tends to have reduced activation energy, leading to high catalytic efficiency, which may possibly be attributed to an enhanced local or overall flexibility of the structure of the protein. Membranes appear to incorporate specific lipid constituents to maintain fluidity and the critical ability to transport substrates and nutrients under very cold, otherwise rigidifying conditions. Cold-shock proteins enabling specific continued activities as the temperature drops are induced in psychrophiles, as in other thermal classes of microorganisms. With limited information from a small number of 'model' organisms, however, these potentially universal themes for defining cold adaptation have been embraced only cautiously in the literature. This situation is poised to change, as the focus on microorganisms in very cold habitats sharpens and the research enterprise on the subject of cold adaptation broadens (for recent reviews, see [1–6]).

In this brief review of the most recent work on psychrophiles, selected advances at the molecular level are highlighted, along with parallel advances made in naturally cold habitats at the level of whole microbial organisms, populations and communities. In the latter case, the main focus is on very cold, typically subzero, aquatic environments,

including the deep sea (-1°C to 4°C), Arctic and Antarctic marine habitats (seawater and sediments near -1°C and sea ice, where internal fluids remain liquid to -35°C in winter-time), and glacial and lake ice (down to -5°C). The polar regions of the Earth, especially the Arctic, are undergoing relatively rapid environmental change on a global perspective [7], such that inhabitants dependent on the frozen environment of sea ice appear threatened with extinction in this century. Motivations to document biodiversity at high latitudes, especially in ice, are strong, just as the new field of astrobiology demands research in Earth's frozen polar regions as habitat analogues for possible life elsewhere [8,9]. The most likely candidates for harboring microbial life in our solar system, now or in the past, are Mars, with its present polar ice caps and indications of past water [10], and Europa, Jupiter's ice-covered moon with a possibly vast ocean below the ice [11]. Both bodies present us with frozen surfaces for evaluation and sampling.

Advances at the molecular genetic level

Most research at the molecular genetic level in cold-adapted microorganisms continues to focus on enzymes, although other cellular constituents are also receiving attention now (Table 1). Joining the list of enzymes already known and at least partially characterized from cold-adapted bacteria are the first xylanases and laminarases [12], valine dehydrogenase [13], chitinase and chitinases [14,15,16], and pectate lyase [17]. Increasingly sophisticated work with some of these and with previously characterized enzymes have reinforced the general themes of cold activity, especially that key adaptations in the form of specific amino-acid residues are located in the domain of the active site or, if distant, nevertheless influence the conformational flexibility required of the site for reactivity in the cold [13,15,18]. The expression of genes for enzymes from cold-adapted microorganisms has been advanced through the use of conventional (*Escherichia coli* [16,18–20]) and novel vectors, including the first recombinant protein produced in an Antarctic bacterial host [21]. The traditional approach of using mutants to understand gene expression has been put to good advantage in evaluating the number of evolutionary steps that may have been required to achieve cold activity in an α -amylase [22]. More research has also been directed towards understanding how an extracellular enzyme behaves in a very cold environment, once released by the producing organism, and thus the importance of enzyme lifetime in the cold [2,9,23]. The possibility of co-evolutionary processes contributing to enzyme behavior in a cold environment has also been recognized, in the form of vertebrate (fish) host control of bacterial enzyme production [24].

Advances with proteins other than enzymes include new work with elongation factor 2 proteins from an Antarctic methanogen [25], addressing some of the cold-active

Table 1

Understanding cold adaptations at the molecular genetic level.			
Organism source	Targeted cellular constituent	Selected findings	Reference
Antarctic Bacterium, <i>Cytophaga</i> sp KUC-1	Valine dehydrogenase	Amino acid residues implicated in conformational flexibility for cold activity	[13]
Icelandic Bacterium strain PA-43	Alanine, malate dehydrogenases	Typical cold-active enzyme traits	[18]
Arctic Bacterium, <i>Colwellia psychrerythraea</i> strain 34H	Leucine amino-peptidase	Lowest temperature for optimal activity of an extracellular protease	[23]
Fish pathogenic Bacterium <i>Flavobacterium psychrophilum</i>	Metalloprotease	Environmental host factors governing bacterial enzyme production	[24]
Antarctic Bacterium <i>Pseudoalteromonas haloplanktis</i>	β -galactosidase	Typical cold-active enzyme traits faithfully expressed in <i>Escherichia coli</i>	[19]
Marine Bacterium <i>Moritella marina</i> MP-1	β -lactamase	Heat lability of enzyme retained when expressed in <i>E. coli</i>	[20]
Antarctic Bacterium	α -amylase	Many evolutionary steps to cold activity evaluated using mutants	[22]
Antarctic Bacterium <i>P. haloplanktis</i> TAC125	Plasmid pMTBL, α -amylase	First recombinant protein production in an Antarctic host	[21]
Antarctic Bacterium <i>Arthrobacter</i> sp. TAD20	Chitinases	Cold activity on both dissolved and particulate substrate	[14]
Antarctic Bacterium <i>Arthrobacter</i> sp. TAD20	Chitobiase	Key cold adaptations located in domain of active site	[15]
Antarctic Bacterium <i>Vibrio</i> sp strain Fi:7	Chitinase	Cold activity faithfully expressed in <i>E. coli</i> , doubled by Ca^{2+}	[16]
Antarctic Bacterium <i>P. haloplanktis</i> ANT/505	Pectate lyase	First pectate lyases characterized from cold-adapted marine bacteria	[17]
Antarctic Archaeon <i>Methanococcoides burtonii</i>	Elongation factor 2 proteins	Cold-active traits of overproduction, greater affinity for GTP, and stabilization by the ribosome	[25]
Bacterium <i>Listeria monocytogenes</i> LO28	Cold-shock proteins	Enhanced survival at low temperature and high pressure	[26]
Bacterium <i>Arthrobacter agilis</i>	Carotenoids	Carotenoids contributing to membrane stability at low temperature and high salt	[29]
Arctic Bacterium, <i>C. psychrerythraea</i> strain 34H	Whole genome	Amino acid substitutions important to cold adaptation, based on comparative analyses of first psychrophilic genome with mesophilic genomes	[32]

features of the protein-synthesizing machinery (Table 1), and additional research on cold-shock proteins [26]. In the latter case, links between cold-shock proteins and markedly enhanced survival of the producing bacterium under elevated hydrostatic pressure reinforce expected links between psychrophily and barophily in general [27]. The most recent discovery of Bacterial genes for cold shock proteins in some Archaea, the uncultured cold Crenarchaeota from Antarctic waters [28], contributes to the discussion of environmentally driven gene transfers across phylogenetic domains. Adaptations to other forms of environmental challenges experienced at low temperatures, such as salt stress, via the production of carotenoids [29], suggest that research on carotenoids (and perhaps pigments in general) in bacteria from the briny pockets of sea ice may reveal new links between psychrophily and halophily (or halotolerance). Indeed, in sea ice, a decrease in temperature (such as occurs during winter) necessarily means an increase in salt concentration in the remaining fluid of the ice matrix [2,30], yet this particular dual-factor challenge to the resident bacteria is often overlooked [31].

Regarding the essential cellular constituent, researchers at The Institute for Genomic Research have nearly completed

sequencing and annotation of the first whole genome for a cold-adapted organism [32] (Table 1), the obligately psychrophilic protobacterium *Cokwellia psychrerythraea* strain 34H. This organism was isolated originally from Arctic marine sediments [23] but is known to have many very close relatives, cultured and uncultured, throughout Arctic and Antarctic polar environments, especially in sea ice [33,34]. Indeed, it represents the only genus for which all known (cultured) members are psychrophilic [35]. The availability of this genome thus provides an important new database and range of approaches for analyzing a broad spectrum of cold adaptations, especially in the marine realm. Preliminary comparative genome analyses of *C. psychrerythraea* with related protobacterial but mesophilic genomes have already underscored the importance of amino acid composition and the predominance of specific residues for cold activity [32].

Microbial diversity in polar and cold deep-sea environments

With the advent of molecular phylogeny and methods for assessing unculturable microorganisms in the environment came the initiation of biodiversity surveys based on the 16S rRNA gene sequence. Although cold regions were not

Table 2

Describing microbial diversity in polar environments.

Environment	Approach*	Selected findings	Reference
Pacific Ocean waters, including cold deep sea	Quantitative culture-independent survey of Bacteria and Archaea	Archaea, especially Crenarchaeota, dominant in cold deep sea	[36**]
Arctic seawater and nepheloid (particle-rich) layers	Quantitative culture-independent survey of Bacteria and Archaea	Bacteria, especially CFB, dominant in surface waters; Archaea elevated in the deeper nepheloid layers	[38]
Arctic marine surface sediments	Quantitative culture-independent survey of Bacteria and Archaea	High fraction, especially sulfate-reducers and CFB, of diverse Bacteria; low fraction of Archaea, limited diversity	[40]
Deep Antarctic seawater	Culture-independent survey of Bacteria and Archaea	Diverse Bacteria, γ -proteobacteria dominant; Archaea limited to Euryarchaeota	[39]
Antarctic lake microbial mat	Culture-based and culture-independent surveys of Bacteria and Archaea	Diverse Bacteria; limited diversity of Archaea	[37]
Antarctic and Arctic sea ice	Culture-independent survey of Bacteria and Archaea	Diverse Bacteria, with some common to both poles; no Archaea	[33]
Deep cold Pacific Ocean waters	Quantitative culture-based survey of salt-tolerant organisms	Cold-adapted halotolerant <i>Halomonas</i> in high numbers	[44]
Arctic sea ice	Quantitative culture-based survey of cold-adapted facultative oligotrophs	Diverse Bacteria, including novel organisms; high culturable percentages and low diversity in upper ice horizons	[34]
Arctic seawater	Culture-independent survey of Bacteria	Diverse Bacteria, including novel organisms; depth-related and seasonal patterns	[69]
Antarctic and Arctic seawater	Culture-independent survey of nitrifying bacteria	Ammonia-oxidizers at both poles, but hints of endemism	[43*]
Antarctic and Arctic seawater	Culture-based survey of cold-adapted facultative oligotrophs	Diverse bacteria among the 173 isolated strains	[70]
Antarctic seawater (and deep waters elsewhere)	Culture-independent survey of Archaea	Euryarchaeota groups II, III and IV present, but haloarchaeal group IV only in deep waters	[41]

*All of these studies, whether culture-based or culture-independent, rely upon analyses of 16S rRNA gene sequences. CFB, *Cytophaga-Flavobacterium-Bacteroides* phylum within the Bacteria.

the first environmental targets of interest, they are now receiving increased attention (Table 2). When both Bacteria and Archaea have been surveyed across a range of polar and other cold environments [33,36**,37–40], the Bacteria are observed to dominate and be present in greater diversity than the Archaea. The important exceptions are the cold interior and deep waters of the ocean, well below the upper mixed layer. There, the Archaea appear in numbers elevated above their counterparts in surface waters [38,41] or actually higher than the Bacteria at the same depths [36**]. In the latter case in the deep Pacific Ocean [36**], the populations of Crenarchaeota (when added to the Euryarchaeota that were also present) largely accounted for the Archaeal numbers matching or exceeding the Bacterial numbers. Crenarchaeota are also found in deep Antarctic waters [28], though only Euryarchaeota groups were detected among the Archaea in a recent study [41]. The deep Arctic Ocean remains entirely unexplored from this perspective. Because the study of Archaeal diversity in polar and other cold environments is still in its infancy, use of the phrase ‘limited diversity’ in Table 2 is best taken with a grain of salt.

Archaea can be tracked in time and space not only by 16S rRNA gene surveys but also via their unique lipid signatures. These features have made possible the dating of the expansion of the Crenarchaeota from high-temperature environments (where they were first detected and thrive today) to the cold deep sea [36**] (and elsewhere) from analyses of deep-ocean drilling cores [42*]. The expansion

appears to have occurred during the mid-Cretaceous period, over 100 million years ago and, on the basis of stable carbon isotope analyses of the same core samples, to have been dominated by a chemolithotrophic lifestyle. Given this information and the chemolithotrophic nature of culturable nitrifiers, the documentation of Bacterial nitrifying genes in cold oceans [43*], the evidence for lateral gene transfer between Bacteria and contemporary cold-ocean Crenarchaeota [28*] and various correlations between physical–chemical hydrographic and Archaeal (and other microbial) parameters in the Northwest Passage of the Arctic Ocean [38], the currently unknown metabolic functions of the cold Archaea are suggested to include nitrification (LE Wells, JW Deming, unpublished data). If true, the cold Crenarchaeota will enter center stage, not simply for their elevated numbers in cold waters, but for their participation in the nitrogen cycling process considered responsible for continued primary production throughout the ocean.

Continued studies of the various species, genera and phyla of the Bacteria that are present in a range of polar environments (Table 2) indicate that even this better-explored phylogenetic domain holds surprises in store. Conventional quantitative cultivation methods, newly applied to salt-tolerant organisms in the cold deep sea [44] and to psychrophilic bacteria in sea ice [34], have yielded many isolates with novel 16S rRNA sequences that grow at -1°C . They have also revealed that culturable organisms can comprise surprisingly large fractions of the total populations, especially in sea ice [34].

Table 3

Evaluating microbial dynamics in polar environments.

Environment	Measurements	Selected findings	Reference
Arctic, Antarctic, temperate and tropical seawater	Analyses of published data on bacterial growth efficiency and temperature	Low fraction of assimilated carbon <i>respired</i> at high latitudes, [48] more carbon available for export	
Antarctic seawater (Ross Sea)	Oxygen utilization, ³ H-leucine incorporation	Rates of <i>respiration</i> critical to estimating bacterial carbon demand	[71]
Antarctic seawater (Ross Sea)	Concentrations and fluxes of glucose, dissolved polysaccharides	Rapid post-bloom degradation of labile polysaccharides that first accumulated during the phytoplankton bloom	[49]
Antarctic lake water	Bacterial production and respiration, DOC inputs	Discrepancy between bacterial <i>respiration</i> and DOC supply indicative of missing carbon sources	[72]
Arctic seawater (North Water polynya)	Bacterial abundance, actively respiring fraction, extracellular enzyme activities, POM fluxes	Actively <i>respiring</i> particle-associated bacteria depleting via protease activity the nitrogen content of fluxing POM	[50]
Arctic marine sediments	Cell-specific sulfate-reduction rates, sulfur-isotope fractionation	Temperature-dependent shifts in types of sulfate- <i>respiring</i> organisms	[73]
Arctic seawater (Chukchi Sea)	Bacterial abundance, size, actively respiring fraction, substrate uptake, rRNA-based community structure, viral density	Peak in viral density coincident with shifts in bacterial size, numbers, <i>respiratory</i> fraction, community activity and structure	[51]
Arctic seawater (North Water polynya)	Bacterial and viral abundance, ³ H-thymidine incorporation, batch-culture experiments	Active microbial loop with significant role for viruses; widespread, cold-adapted virus–host system obtained	[52]

DOC, dissolved organic carbon; POM, particulate organic matter.

Although environments exposed to the coldest of temperatures (such as the upper horizons of Arctic sea ice, which can experience temperatures to well below -20°C in winter [45**]) do appear to support the lowest observed diversity of Bacteria [34], the rarity of studies of natural environments at any temperature below that of polar seawater (-1.7°C) makes any conclusion about temperature-limited diversity at the low end of the temperature scale premature. More work on the coldest of microbial habitats — Arctic sea ice and Antarctic salt lakes in the ‘dead’ of winter [46] — is needed to answer this question.

Microbial dynamics in polar regions

Although molecular methods are expected to allow an assessment of microbial dynamics as well as diversity in a given environment, understanding the functional roles of microorganisms in the cycling of carbon, nitrogen and other elements and materials in a very cold ecosystem continues to rely upon more traditional methods, albeit applied with new insight. Recent applications of radiolabeled tracer techniques for measuring microbial activity in polar regions, or new evaluations of previously published data, have tended to focus on the issue of bacterial respiration (Table 3). Respiration appears to diminish in accordance to growth at increasingly cold temperatures. This conclusion was reached in a recent dissertation [47] on the basis of field measurements in the Arctic Ocean (in the Northeast Water polynya off Northeast Greenland) and related conceptual and quantitative modeling efforts, but placed into global context in a recent article [48]. Increased bacterial growth efficiency at high latitudes translates into increased availability of particulate organic matter (POM) for export to depth, placing cold-driven patterns of microbial metabolism squarely in the arena of global carbon sequestration and climate change.

As emphasized in a recent review [31], however, the control of heterotrophic microbial activities by temperature can rarely, if ever, be understood separately from the issue of available food in the form of dissolved organic matter (DOM). Some advances have been made with regard to the sources, types and microbial fate of both DOM and POM in polar marine settings (Table 3). Labile polysaccharides stemming from Antarctic phytoplankton blooms in near-freezing waters appeared to be readily consumed by the bacterioplankton, albeit after first accumulating undegraded during the bloom [49]. This pattern is in keeping with the concept of a higher threshold requirement for DOM by bacteria operating at suboptimal growth temperatures [31]. POM, the ultimate source of DOM, was observed to diminish seasonally in its nitrogen content in an Arctic polynya (the North Water in northern Baffin Bay), probably because of the hydrolytic actions of extracellular proteases produced by the fraction of particle-associated bacteria that were actively respiring [50].

Rare studies combining molecular methods and traditional abundance and activity measurements reveal an important role for viruses in the production and fate of DOM and the successional dynamics of Arctic bacteria in the Chukchi Sea, north of Alaska [51*], in the North Water [52] (Table 3) and in the Northwest Passage [53]. A marine virus–host system, unique in its cold adaptation and widespread in regional occurrence, was recently obtained from the highly productive ecosystem of the North Water [52]. Its availability portends well for future advances not only in understanding the dynamics of polar microbial ecosystems and carbon cycles but also in exploring lateral gene transfer at cold temperatures.

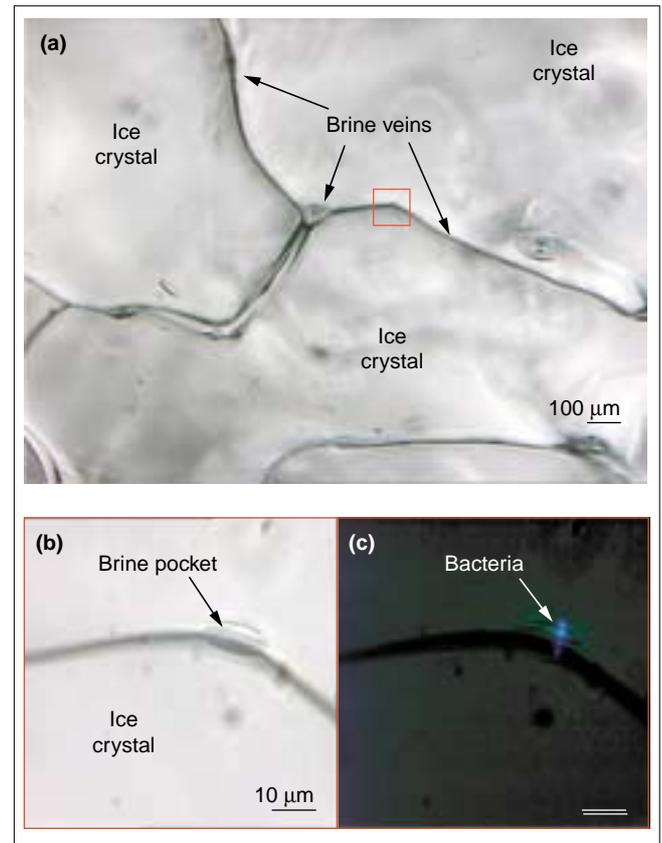
A surprising gap in knowledge of microbial strategies for acquiring adequate food (or avoiding negative conditions) in cold regions concerns chemotaxis, the movement up a gradient of utilizable substrate or chemical attractant (or down one of repellent). Chemotaxis enables a microorganism to position itself favorably for competitive growth and survival. Although the temperature of the vast portion of the global ocean, and virtually all waters at high latitudes, lies below (often well below) 5°C, and much is made of the importance of chemotaxis in the sea [54], virtually nothing is known about the ability of bacteria to adopt chemotaxis as a survival strategy in the cold. Like the virology of the cold ocean [51,52,53], this void will soon begin to fill with much positive information [55].

Microbial life in frozen environments

Ice, whether in the form of tundra, glacier, snow, lake or sea ice, presents a special environment for microbial life [2,9,30,56,57]. It arguably provides the more selective environment for cold adaptation than milder unfrozen bodies of water or sediment. The seasonal process of encasement of marine microbial communities into sea ice, exposure to severe winter conditions and release again in summertime is postulated to seed the global ocean with psychrophiles annually ([2,45**] and citations therein). Continued activity during wintertime would ensure a positive selection process for cold adaptation rather than only a destructive one against freeze-intolerant organisms. Indeed, the lower temperature limit for metabolic activity (as opposed to preserved life) is sought in various forms of ice, particularly freshwater forms, because of astrobiology-defined interests in habitat analogues for soil-covered Mars (Arctic tundra) and ice-covered Europa (Antarctica's Lake Vostok buried deep below glacial ice) [8,56,58]. Theoretical considerations of deep glacial and lake-ice matrices imply adequate energy resources for bacteria and room for their movement in the veins between ice crystals that remain liquid-filled at the *in situ* temperatures relevant to these environments (down to -5°C) [56]. Incubation experiments using tritiated thymidine and leucine with Antarctic snow-ice samples, which experience colder atmospheric temperatures, have indicated metabolic activity to -17°C [59]. Other research with frozen samples of Siberian tundra, first slurried (slightly warmed) to amend with ¹⁴C-acetate, has pushed the lower limit for detectable activity to -20°C [60].

Until recently, interest in sea ice as a natural medium for exploring the lower temperature limits for microbial activity has been limited by comparison [45**,46,57]. This delayed interest is perhaps surprising, given the extreme temperatures to which the upper horizons of sea ice are exposed during wintertime, the capacity for brine networks in Arctic sea ice to remain liquid to -35°C, and the presence of ample organic resources in that network [61]. Advances are coming quickly, however, as researchers develop novel microscopic means to examine bacteria directly within the matrix of (unmelted) winter sea ice for the first time

Figure 1



Bacteria visualized microscopically by DAPI (4,6-diamidino-2-phenylindole-2HCl) stain directly within a brine pocket of Arctic winter sea ice at -15°C. The transmitted light image in (a) shows ice crystals and the brine-filled veins between them (bar = 100 μm). Enlarged images (bar = 10 μm) of a brine pocket in (a) show, (b) by transmitted light, the microscale habitat and, (c) by epifluorescence microscopy, its bacterial inhabitants. Adapted from Junge *et al.* [45**].

(Figure 1) [45**], document respiratory activity and presumably competent protein-synthesizing machinery (as reflected by the fluorescent *in situ* hybridization [FISH] method for quantifying microbial diversity) to -20°C [46], and uncover novel means of cryoprotection in the form of high concentrations of exopolymeric substances (EPS; [62]; C Krembs, H Eicken, K Junge, JW Deming, unpublished data). EPS as cryoprotectants add to the discovery of microbially produced ice-active substances that serve a similar role in Antarctic photosynthetic mats [63]. Also clear, however, is the absence of a need for specific antifreeze compounds when the inhabitable space within ice becomes limited to the size of the bacterium itself. At that point, the known ordering behavior, surface charge and polarity of water molecules in confined space alone can account for the persistence of the liquid phase [64]. The inevitable presence of some liquid even within the coldest of natural ice formations promises more surprises regarding the lower temperature limit for microbial life and the mechanisms supporting it.

Eutectophiles: life at the phase change of water to ice

The newly defined field of astrobiology has stimulated many researchers to think in new ways about the possible limits of life. Instead of restricting theory and experiments to the nature of existing habitats on Earth, more extreme environmental conditions elsewhere are under consideration. An unavoidable extreme in the search for life elsewhere is that of severely cold temperature [9]. At what point does ice fail to support life? Even the coldest forms of ice retain liquid water within their matrices, between individual ice crystals, wetting each crystal surface. The coldest ice forms so far examined (sea ice at -20°C) contain living bacteria within this liquid phase of the ice [45••,46]. Forms of ice not found on Earth, such as ice-VI, but that can be created in the laboratory under enormously high pressures (and surprisingly warm temperatures) are now observed to support living bacteria as common as *E. coli* [65••]. For the case of life in ice-VI (in which the solid phase of water is heavier than the liquid phase), as for the case of Earthly ice (ice-I, for which the reverse is true), microorganisms are observed microscopically at the interface between crystal and liquid (Figure 1).

This eutectic interface between the solid and liquid phases of water is anything but a dead zone and may well be a biochemical crucible for life. Freezing has recently been shown to improve stereoselection (L-enantiomer amplification) in the case of L- and DL-leucine co-oligomerization [66••], as well as enhance the rate or yield of other biomolecular reactions [67]. The eutectic phases of ice — the ice matrix with its concentration of solutes in the spaces between ice crystals — have been used successfully as the reaction medium for synthesizing oligonucleotides non-enzymatically [68••]. New terminology for organisms that take advantage of this critical interface between solid and liquid phases of water is in order: 'eutectophile' is proposed. The term is unusual in that it does not classify an organism artificially by a single parameter (as in the case of psychrophile, barophile and halophile, for example) but more realistically, by whatever known and as yet unexamined combinations of temperature, pressure and solute concentrations that provide a habitat where both the solid and liquid phases of water, and the chemical and biomolecular reactions that occur in that space, are available to the organism for continuation of its life processes. 'Eutectophile' points the way to a new class of experiments already underway, as indicated in this brief review.

Conclusions

The steady increase in characterizations of enzymes, other proteins and membrane constituents and the genetic regulatory mechanisms that enable an organism to live in the cold has lent weight to the guiding principles already established, if tentatively, for cold adaptation. These principles, especially the favoring of specific amino-acid residues that impart flexibility to the active domain of an enzyme molecule, are advanced by the availability of the

first whole-genome sequence for an obligately psychrophilic bacterium, *C. psychrerythraea* strain 34H [32]. The ability to search for other cellular constituents and characteristics, especially non-catalytic proteins and pigments for surviving cold, salt and pressure shocks, seems particularly promising.

The increased application of molecular techniques based on 16S rRNA genes to polar and cold deep-sea environments has resulted in a young but substantive database on the diversity of Bacteria and Archaea, and subgroups within each domain, inhabiting very cold waters, sediments and ice. The answer to the central question of microbial diversity at low temperatures has moved rather quickly from 'not addressed' to 'relatively limited' to 'diverse' [69•]. The future must reveal how diverse, relative to warmer habitats. The answer will have significant implications for the response of polar ecosystems to environmental change, especially in the form of warming, and to the potential rise in the importance of microbial activities and as yet unpredictable shifts in microbial-driven biogeochemical cycles.

Special focus on frozen natural environments, from tundra to sea ice, has yielded new methods for examining microorganisms undisturbed in their native frozen habitats and new ways of thinking about the limits of microbial life. That favorable reactions and life processes go forward at the critical interface of the water-ice phase change, even under temperature and pressure combinations not encountered on Earth [65••], has been recognized. The startling concept of ice forming at high temperatures off Earth and supporting thermophiles now seems within the realm of possibilities. The new field of astrobiology has, thus, required advancing terminology beyond psychrophiles and other single-parameter classifications to eutectophiles, acknowledging the critical interface between solid and liquid water, wherever it forms, for microbial life.

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