STRESSORS, STRESS AND SURVIVAL; OVERVIEW

Everly Conway de Macario, and Alberto J. L. Macario

Wadsworth Center, New York State Department of Health; and Department of Biomedical Sciences, School of Public Health, The University at Albany (SUNY); Albany, New York, USA.

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1. ABSTRACT

This overview introduces the contributions in this Special Issue with the aim of presenting an integrated picture of it. The contributions cover several important areas: protein stability and function under extreme conditions, osmotic stress and osmoadaptation, the structural features of the cell membrane and their possible significance with regard to heat stress, the molecular chaperone machine and multicellular structures as anti-stress mechanisms, peptidyl-prolyl *cis-trans* isomerases, proteases and the proteasome, and oxidative stress and the role of superoxide dismutase. These topics are briefly discussed to explain the basic concepts underpinning them, quoting for the most part introductory articles or reviews that might help the non-specialist to become familiar with the central themes of the Special Issue. As mentioned in the Preface every effort has been made to discuss the archaeal features within the context of other disciplines and biology in general, against the background of what is known for bacteria and eucarya. Hopefully, this approach will help the reader in understanding what is unique to the archaea, what is shared between them and the members of the other two phylogenetic domains, and how studies in archaea impact on other fields of science.

2. STRESS AND PROTEIN STABILITY AND FUNCTION

The article by Scandurra *et al.* deals with the difficult topic of protein stability and function under conditions that would be stressful, or even lethal to humans. Two questions come to mind. Why is the topic difficult? is one of them. The answer is: because it is not readily apparent how molecules can survive and work at temperatures, or pH and salinity levels, etc., that, according to classical wisdom, would seriously damage biological structures in most of the common species of mammals whose body temperatures are close to 37 °C.

The second question stems from the fact that any conditions different from those that are optimal for human cells are called "extreme." Why are they so called, if there are many organisms for which these conditions are optimal? A discussion of the reasons for the usage of the word "extreme", "extremophiles", and related terms is beyond the scope of this Overview. Suffice it to say that those terms probably are a reflection of a human-centered culture that places human beings at the "center of creation." However, we now know enough of the variety of life on Earth to acknowledge that there are organisms capable of growth across a wide range of conditions (1). This notion is of fundamental importance in the definition of stress, which is a situation caused by stressors (2). These are changes in the conditions under which an organism normally lives. Stressors are agents of a physical, chemical, or biological nature that represent a change in the usual environmental conditions for any given life form. It follows that while a specific condition (*e.g.,* a temperature of 65 °C) may be stressful (or even lethal) to a certain species that normally lives at 37 °C, it will be optimal for growth to a thermophilic organism. (See Preface for definition of terms used throughout this article).

What are the structural features that enable proteins to survive and function in thermophilic organisms, some of which—called hyperthermophiles—have optimal temperatures for growth (OTG) near or above the water-boiling temperature? A recent article describes an extensive analysis of proteins in databases (64 from mesophilic and

29 from thermophilic organisms) in search of structural features that would distinguish mesophilic, moderately thermophilic, and extreme thermophilic molecules from one another (3). The properties examined were molecular cavities, hydrogen bonds, ion pairs, secondary structures, and polarity surfaces. As in earlier studies, the data did not show dramatic differences among the three groups of proteins. The only parameter that showed some distinctive difference was the number of ion pairs, which increased in parallel with the OTG of the organisms studied. A comprehensive review on the topic is offered in the article by Scandurra *et al.* The main factors involved in the study of protein stability and function are explained. The authors also present a discussion of thermodynamic and structural aspects of protein stability and conformational fluctuations in response to environmental changes.

The most remarkable part of the answer to the protein stability question is that it depends upon the sum of very many minute factors rather than upon a major, easily observable structural feature or set of features.

Thermodynamically, free energy changes due to the interplay of stabilizing and destabilizing forces are quite limited, within the range of 5-17 kcal/mol, for proteins from mesophiles, as well as for proteins from hyperthermophiles. Adaptation to high temperature in extremophilic proteins is associated with free stabilization energies like those of mesophilic molecules.

Proteins from hyperthermophiles have apparently evolved to reach a compromise between high stability (rigidity) and preservation of enough flexibility to allow for the changes in shape required for function. Rigidity is mediated by weak interactions (van der Waals, electrostatic, and hydrophobic) whose cumulative effect is the required stability. Proteins from psychrophiles are more rigid than those from hyperthermophiles, but the stabilizing forces are of the same type and, individually, of the same magnitude.

Some amino acids (aa) seem to be preferred by hyperthermophilic proteins, such as charged ones. In regard to their three-dimensional arrangement, hyperthermophilic molecules display more hydrogen bonds than mesophilic counterparts.

Proteins from extreme halophiles show a preference for acidic over basic aa, and have more serine than threonine residues than the mesophilic equivalents. Negatively charged aa abound at the protein surface, which makes a sort of protective shell against the high-salt surroundings.

3. OSMOTIC STRESS

Adaptation to high salinity, and the response to hyper- and hypo-osmotic shocks are treated in the article by Roberts. Essentially, two types of response are described: short- and long-term, as explained in the Preface. Each has a different set of molecules as distinctive players, whose role is to maintain a physiological concentration of

intracellular proteins with a functional configuration. These distinctive players must also maintain a physiological concentration of electrolytes, and cell volume and turgor, all so important for life.

A common mechanism for counteracting an increase in the external salinity is the intracellular accumulation of compatible solutes, so called because they do not interfere with cellular functions despite their high concentrations. These and other related biochemical events have been extensively studied in bacteria, and in eukaryotes such as the yeast *Saccharomyces cerevisiae.* In a recent review, the yeast's strategies to cope with osmotic stress and avoid dehydration were presented to show that the mechanisms involved are complex, and include also metabolic pathways other than those leading to the generation of compatible solutes (4). The yeast cell excludes the extracellular stressor (excess NaCl) and accumulates compatible solutes, such as glycerol. In addition, the authors report, there is induction of genes involved in glycerol dissimilation and trehalose turnover.

In contrast, the response to a decrease in environmental salinity includes the reduction of osmolytes, which may be accomplished by enhanced efflux and/or catabolic elimination. One question that remains unanswered, as explained in the article by Roberts, is whether or not archaea possess homologs of two osmoadaptation mechanisms present in bacteria: mechanosensitive ion channels (MSC) and volume-activated channels (VAC). The answer seems to be negative, at least at the present time. There are no identifiable MSC or VAC candidates in the archaeal genomes fully sequenced thus far. However, the existence of solute-expulsion mechanisms in archaea cannot yet be ruled out. More research is needed to clarify this critical aspect of the archaea's adaptation to high-salt environments, since many species live in these environments.

A few archaeal species have been found to have the capacity for accumulating exogenous osmolytes such as betaine. Likewise, some archaeal species can accumulate K^+ in a manner dictated by external salinity, while in other species intracellular K^+ levels are not perturbed by external salinity changes.

Organic solutes also play a role in the response to osmotic shock of another archaeal group, the methanogens. The few methanogenic species studied thus far were found to produce aa-like molecules that were non-reactive with intracellular components (*i.e.,* they were compatible solutes). In addition, stress proteins may also play a role in osmotic stress inasmuch as they prevent protein unfolding and promote refolding of partially unfolded polypeptides.

4. CELL MEMBRANE

Adaptation to high temperature or salinity, for example, requires a number of intracellular mechanisms as described in the previous subsections, and it also involves the cell membrane. The role of the cell membrane in stress

resistance is discussed in the contribution by Albers *et al.* The authors summarize the structural features of the archaeal-membrane lipids and contrast them with those of the bacterial membrane. For example, the variety of the hydrocarbon moiety of the archaeal-membrane lipids is presented as a basis for adaptation to stressful conditions. It has been known for quite some time that one of the characteristics distinguishing archaea from bacteria is the structure and composition of the membrane lipids. If one considers the critical role of lipids in maintaining membrane integrity and function, it is conceivable that archaea have developed unique sets of molecules, different from those found in bacteria, to endow their cell membranes with the properties necessary to function in their various ecosystems. This is, in fact, an area ripe for investigation aimed at discovering novel mechanisms for membrane assembly, maintenance, and transport in archaeal species from different ecological niches.

In archaea, branched hydrocarbons of the phytanyl or biphytanyl type are linked to glycerol, or variations of it, via ether bonds. This is in contrast to bacteria, in which glycerol is linked to fatty acids via ester bonds.

It is believed that the comparatively high stability of the archaeal membrane lipids in the face of an elevated temperature, for example, is due to a reduced mobility of the phytanyl chains caused by the methyl group that occurs every fourth C atom in the chain's backbone. This reduced mobility would increase heat resistance and decrease membrane permeability. Low permeability to protons would increase resistance to oxidation and to high temperature, both resistances depending mainly on the ether type of linkage. Furthermore, this type of bond would also be responsible for the very low susceptibility of archaeal membranes to degradation at high pH (saponification), and to digestion by phospholipases. These are the properties that make liposomes made of archaeal membranes very resistant in comparison to those made of bacterial membranes, a property that confers on archaeal liposomes practical advantages over the bacterial equivalents (5, 6).

Adaptation to high temperature during evolution has apparently resulted in changes that favor maintenance of a fluid membrane while reducing proton loss. This is partly achieved by cyclization of a long isoprenoid chain that results in its tight packing. These structural features do not seem to have a visible effect on Na^+ transport, though. In eukaryotes, membrane sphingolipids are involved in the mechanism of ubiquitin-dependent proteolysis caused by heat stress (see Section **7**).

In addition to lipids, there are several proteins in the archaeal membranes, which make up 50% or more of these membranes. Unfortunately, very little is known about membrane proteins in archaea. Some are members of the ABC-transporter family as reported for the first time in 1996 (7). Genome sequencing has later confirmed and expanded those original findings (8). It has also been found that a family of membrane proteins from the methanogen

Methanosarcina mazeii S-6 possesses tandem repeats (9, 10). The repeats are probably the basis of antigenic diversity and variation. The data suggest that *M. mazeii* has evolved a mechanism for changing its surface as required by environmental modifications, by gene rearrangement and module exchange with the potential to generate a large array of different proteins using a relatively limited amount of building blocks (the repeats) (11). It would seem that the capacity for assembling a variety of cell surfaces originated independently, without connection to a need to escape immune surveillance, since *M. mazeii* S-6 is not a pathogen and does not have to deal with antibodies or lymphocytes from a host. It must be said, however, that the possibility that *M. mazeii*, and other methanogens, inhabited the intestinal cavity, for example, of pre-historic animals cannot be ruled out. Perhaps they were forced to develop a capacity for antigenic variation in these pre-historic hosts, and not only as a reaction to environmental changes unrelated to immune mechanisms of larger living beings.

5. MOLECULAR CHAPERONES AND ANTI-STRESS MECHANISMS

The central event unchained by a stressor impacting on a cell is protein denaturation, which in turn elicits the stress response (2). As seen above, this response involves the cell membrane and intracellular mechanisms. Among the latter is the increase in the stress or heat-shock proteins (Hsp), including molecular chaperones whose central role is to assist in the folding and re-folding of polypeptides, as they are produced in the ribosome and as they are unfolded because of the stress, respectively. Molecular chaperones are a means to abate irreversible protein denaturation. Hsp belong to several families according to their molecular mass (12). An important family is constituted of the Hsp70(DnaK) molecules. Hsp70(DnaK) forms the molecular chaperone machine by associating with members of the Hsp40(DnaK) and the small heat-shock protein (Hsp) families in bacteria. The components of the molecular chaperone machine are highly conserved in bacteria and eukaryotes but not as much in archaea: several archaeal species do not have them (13, 14). The distribution and other characteristics of the archaeal molecular chaperone machine are discussed in the article by Macario and Conway de Macario.

Archea also have the chaperonins, which are members of the Hsp60 family (a topic that will be treated in an upcoming article), and have developed what appears to be a variety of means to counteract the effects of stressors and survive in harsh environments. An example of the latter means is the formation of multicellular structures as described in the article by Macario and Conway de Macario.

6. PEPTIDYL-PROLYL *CIS-TRANS* ISOMERASES

Another important family of Hsp, some of which are also chaperones, are the sHsp that have a molecular mass of 34 kDa or less (12). Peptidyl-prolyl *cis-trans* isomerase (PPIase) is one of the members of this family. There are many of them, they play a role in protein folding

in eukaryotes and bacteria, and are present in archaea, as described in the article by Maruyama and Furutani.

Protein folding requires, among other things, rotation of the peptidyl-prolyl bonds. This occurs spontaneously but very slowly. PPIases catalyze this rotation, accelerating it and thus making it compatible with the rapid pace of other intracellular activities.

While all PPIases possess the enzymatic activity that gives them their name, they are not all identical. They can be sorted out into sub-groups considering other properties that are not universal within the family. For example, PPIases are classified into three subgroups according to their ability to bind immunosuppressants. This is explained in the review by Maruyama and Furutani. The authors also describe the methods utilized to assay PPIase activity, and present the data available from experiments *in vitro* and *in vivo*. The few PPIases found in archaeal organisms are treated in detail. Their structure, binding of immunosuppressants, and enzymatic activities are described. In addition, data are presented that help to determine whether or not PPIases have also a typical chaperoning activity, *i.e.*, assistance in the refolding of partially denatured polypeptides.

The study of archaeal PPIases is quite intense because important questions must yet be answered before one can think of how to use these enzymes in industry or therapeutics, for example. Are there representatives of all PPIase subgroups in archaea? Are they present in archaeal species in a seemingly capricious distribution pattern as are the Hsp70(DnaK) proteins? If PPIases also show a discontinuous distribution among archaea, which ones are the most conserved? How many different PPIases co-exist as a rule in archaeal cells? Is there a minimal set of different PPIases that must co-exist in archaeal cells for survival? What do these enzymes do *in vivo*? Do they operate under physiological conditions and also under stress? If the latter is true, do PPIases operate differently during stress as compared with those of unstressed cells? These questions are pertinent to all archaea, but they are particularly challenging in what concerns extremophiles, and finding the answers will have an impact on a variety of fields beyond the archaea.

7. PROTEIN DEGRADATION

Abnormal proteins must either be converted to normality or eliminated, lest they interfere with cellular functions. Abnormal proteins may aggregate, form precipitates, and be toxic, all factors that conspire against cell physiology. Conversion of proteins to normality is mediated by molecular chaperones, whereas elimination of molecules beyond repair is carried out by proteases. A key proteolytic system in eukaryotes includes ubiquitin and the proteasome (15). A protein, or fragments thereof, destined for degradation is tagged by ubiquitin and digested by the proteasome. Proteases and the proteasome are treated in the article by Maupin-Furlow *et al.* It focuses on the proteasome within the context of proteases in general, in the three phylogenetic domains. The relative simplicity of

the archaeal proteasome is contrasted with the complexity of the eukaryotic counterpart. Interestingly, while most bacteria examined do not harbor proteasomes, actinomycetes do. Thus, the 20S proteasome and a related family of molecules named the AAA$^+$ (ATPases associated with various cellular activities) proteins are found in archaea and in the actinomycetes, which are Gram positive. This distribution is a reminder of that of the Hsp70(DnaK) proteins with the absence (deletion) of 23-25 aa in the N-terminal quadrant (16-18). Somehow, methanogens and Gram positive bacteria share characteristics that distinguish them from other archaea and bacteria. This curious relationship between methanogens and actinomycetes is like a Siren's call for scientists who might want to investigate the extent, and evolutionary meaning, of the similarities between these two groups of organisms belonging to different phylogenetic domains.

Proteins in a cell may be normal or abnormal, and both must be degraded at one time or another. Normal proteins are more or less stable and long-lived, depending on their type and role. For example, proteins that regulate gene transcription, cell cycle and division, DNA repair, and metabolic pathways at critical forking points, are needed only temporarily and are short-lived. The cell must be equipped with proteases for the timely elimination of aged proteins and molecules no longer needed. The cell must have means to get rid itself not only of unwanted proteins but also their fragments. Abnormal proteins and their fragments usually tend to aggregate, precipitate, and thereby clutter the intracellular environment. In addition, some abnormal proteins and/or their aggregates are toxic and cause serious diseases (19). It is then not surprising that all organisms are endowed with a supply of protein- and peptide-degrading tools.

There are several causes for the presence of abnormal proteins inside a cell. As we have seen in preceding subsections, stress tends to denature most proteins, even if they are structurally normal. Even in the absence of stress a cell may contain abnormal proteins due to gene mutations, or to deficiencies in the post-transcriptional or post-translational mechanisms. The problem may be compounded when a stressor hits a cell with structurally abnormal proteins, which already have a tendency to aggregate. A combination of stress and genetic or synthetic abnormalities may be deadly (19). Hence, the importance of protein-degradation mechanisms for cell survival cannot be overemphasized.

In eukaryotes, the proteasome is a major cellular tool for degrading proteins that relies on ubiqutin for selecting its targets. Membrane lipids have been implicated in the ubiquitin-dependent proteasome-mediated proteolysis induced by heat shock (20), which indicates once more the importance of the cell membrane in the stress response (see Section 4). Archaea also have proteasomes, but their function and induction mechanisms have not yet been elucidated. The article by Maupin-Furlow *et al.* describes the structure of the proteasome and its component subunits that in archaea are only of two types, or three at most: alpha and beta, or alpha, beta-1, and

beta-2. The subunits are assembled in an alpha7/beta7/beta7/alpha7 circular configuration, forming a cylinder with a central cavity open on both bases and with orifices on the wall.

The authors also describe the mechanism of action of the eukaryotic proteasome, which might reflect that of the archaeal counterpart, although this has not yet been proven. The possible interactions between Hsps and the proteasome are also mentioned as an area for future research. Along these lines, the role of the proteasome in the stress response is also an interesting topic for investigation. Very little is known about the proteasome as an anti-stress tool, but preliminary information suggests that it does play a role that might be important for cell survival.

8. OXYGEN

Temperature, pH, and salinity were mentioned as potential stressors. It is somehow difficult to think that oxygen can also be a stressor. For us, whose life depends on oxygen, it seems improbable that this substance can stress our cells. But it can.

It took perhaps 1.5 billion years for O_2 to reach the 21% level in the Earth's atmosphere, approximately 780 million years ago. No significant changes seem to have happened since then, and today's air has the same percentage of O_2. If life originated 3.5-3.8 billion years ago, it evolved without O_2 (*i.e.*, breathable oxygen) in the atmosphere for a little over a billion years. At this time, it is likely that O_2 began to appear and slowly rise until it reached the current level, which might have happened almost 800 million years ago.

The history of life on Earth may be presented as having gone through two major periods: the first extending from the origins until O_2 levels in the atmosphere reached 21%, and the second period beginning then and extending until today, always with the same level of O_2. Most likely, the transition from the first to the second period was a long process during which all living forms used to anaerobiosis began to be confronted with O_2. The confrontation must have escalated with the passage of time. Many living forms probably became extinct, but others found ways to cope with O_2 and survived, even with its steadily increasing concentrations. Several evolutionary events must have occurred. Essentially, those living forms endowed with a mechanism to use O_2, or at least to defend themselves against the toxic effects caused by it or its derivatives, survived. They became oxygen-respirers (aerobes) or at least oxygen-tolerant. Mechanisms must have evolved to cope with what today are known as the poisonous forms of oxygen, *e.g.,* the reactive oxygen species (ROS). Some of these mechanisms will be mentioned below, and the enzyme involved in one of them, the superoxide dismutase (SOD), is treated in detail in the review by Cannio *et al.*

Aerobic organisms such as most known eukaryotes have mitochondria where O_2 is reduced to H_2O_2 with generation of the energy-rich compounds necessary for the cellular activities. Also, small amounts of toxic forms of oxygen, ROS, are generated in the mitochondria: O_2^- (superoxide), and $OH^\bullet$ (hydroxy radical). In the normal cell, accumulation of toxic oxygen species does not occur because there are mechanisms for their elimination. However, imbalances between ROS production and ROS elimination may happen leading to ROS accumulation, which can cause oxidative stress with damage to proteins, lipids, and nucleic acids. ROS not only cause oxidative stress, which is characterized by activation of some stress genes, but they also repress many genes, as other stressors do (21). This ROS-induced gene down-regulation has profound consequences upon the cell, above and beyond those typical of stress-gene induction. Oxidative stress is, in fact, one of the leading mechanisms of aging and cell death. Thus mitochondria are central players in the cell's life not only because they produce energy from O_2, but also because they have the potential for generating dangerous levels of toxic oxygen derivatives.

Free radicals have an unpaired electron in an outer orbit. The energy generated by this unstable atomic state is released via reactions with surrounding molecules, which results in molecular damage. The mechanisms available to the cell for counteracting the effects of ROS are varied. Examples are: antioxidants (*e.g.*, the lipid-soluble vitamins A and E, ascorbic acid, and glutathione), metals in storage and transport proteins (*e.g.,* transferrin, ferritin, and ceruloplasmin), enzymes that breakdown H_2O_2, and O_2^- (*e.g.,* catalase, glutathione peroxidase, and SOD).

In the article by Cannio *et al*., SOD is treated in detail, particularly in what pertains to its evolution, diversity, and archaeal representatives.

There are several SODs, which form a family of related enzymes with the same catalytic function but with structural differences, that are classified into subfamilies considering their metal cofactors, Cu/Zn, Fe, Mn, or Ni. These subfamilies are described in the review by Cannio *et al.* considering examples from the three phylogenetic domains: Bacteria, Archaea, and Eucarya. The authors focus on the archaeal SODs that have thus far been characterized. Fe, and Mn SODs have been found in hyperthermophilic and halophilic archaea. However, in the halophilic species the SODs are different from other Fe and Mn enzymes known. Curiously, Fe SODs have been discovered in methanogenic archaea that are strict anaerobes. One wonders why these anaerobes that supposedly evolved in ecosystems lacking oxygen have SODs. It has been proposed that the physiological role of SODs in anaerobic organisms might be the reduction of superoxide with generation of hydrogen peroxide, but this remains to be proven.

9. COLD STRESS

In the last decade or so it has become increasingly evident that the limits of life are broader than previously surmised, at least by most people, in terms of the range of temperatures, pH, etc., and the places on Earth in which life forms can thrive. A wide diversity of life

forms has been uncovered, which parallels the diversity of ecosystems in our planet. Some reports even suggest that life might have existed in celestial bodies other than Earth. While this is under scrutiny and a final answer to the question of whether there is, or was, extraterrestrial life may still be years away, it is clear that on Earth microbial life is extremely varied and widespread. For example, today we know that there are microbes that thrive at temperatures above the boiling point of water in contrast to others that live in the polar regions at near-freezing temperatures. Moreover, other life forms inhabit ecosystems with temperatures across the spectrum between 0 and 100 °C. While considerable amounts of information exist about life at temperatures in the mesophilic range, not much is known of life forms that grow well at temperatures above and below it. We also know that a number of stressors, including a temperature elevation above the OTG for a given organism, induce a stress response.

The majority of the biosphere is cold by comparison with the temperature that is pleasant to humans (25-27 °C), or with that which is optimal for human cells in general to grow, divide, and function, namely 37 °C. Hence, we have to assume that there are many forms of life that have OTG below 37 °C. It follows that there is a great deal to learn about life on Earth, *i.e.*, life in cool, or cold environments by human standards. One may assume that the colder the ecosystem, the more different are the molecules and biochemical reactions as compared with those of human cells, or with those of hyperthermophilic organisms. Is this a reasonable assumption? Hints about the right answer to this question will be provided by studying the molecules and biochemical reactions that are characteristic of organisms living in cold habitats such as the Antarctic continent, particularly in what is pertinent to cold acclimation and the cold-stress response.

The cold-stress response has been studied in bacteria representing the Gram positives, *e.g., Bacillus subtilis* (22), and the Gram negatives, *e.g., Escherichia coli* (23). At least three cold-shock proteins have been identified in the former, and nine in *E. coli*. Unfortunately, the biology of organisms that live in cold environments is not as well known as that of these two bacterial models or other life forms that inhabit warmer ecosystems. Data on the cell's response to cold stress are relatively scarce, a situation even more pronounced for the Archaea.

While the classical stress response, namely that induced by stressors such as heat, increase or decrease in pH or salinity levels, chemicals, etc., is characterized by protein denaturation, the cold-stress response is not. Protein denaturation is not a major effect caused by the stressor cold. Other phenomena are considerably more prominent than protein denaturation in the cold-stress response as compared with the heat-shock response. Prominent features of the cold-stress response are: a) Stabilization of the secondary structure of nucleic acids with ensuing inhibition of DNA replication, gene transcription, and mRNA translation; b) Decrease in the activity of many enzymes with the consequent slow-down of metabolism; c) Decrease in membrane fluidity, which tends to impede transport

across it (see article by Albers *et al.*); and d) Formation of crystalline ice, which if unchecked damages intracellular structures and, ultimately, causes cell death.

Evolutionary adaptations to cope with the consequences of cold stress, and to live for long times (acclimation) in cold environments, are directed to counteract the five major effects of cold listed above. For example, psychrophiles have: a) Membranes with special fatty acids that increase fluidity; b) Enzymes constitutively more flexible than the homologs from mesophiles or hyperthermophiles, and c) Anti-freeze proteins that inhibit the growth of ice crystals.

Studies in *E. coli* have shown that the cold-shock response is composed of two phases. The earlier phase is characterized by an increase in the synthesis of cold-shock proteins such as those of the CspA family. The second phase represents an adaptation (acclimation) of the cell to the cold and involves a variety of mechanisms. These and other topics, as they pertain to the archaea, will be clarified by studying archaeal species living in the polar regions, which in turn may help the search for life in celestial bodies with ice.

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