COLD-SHOCK RESPONSE IN MICROORGANISMS

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Summary

This paper describes the effects that cold temperature has on microorganisms and the subsequent cellular responses this provokes. Irrespective of whether a microorganism is a member of the Bacteria, Archaea, or Eukarya, it is unable to regulate its cellular temperature (poikilothermic) and as a result cold permeates every component of the cell. Due to this commonality, all microorganisms studied have a demonstrated ability to respond to the cold by altering their cellular composition using a variety of cold responsive mechanisms. Specific aspects of the cold-shock response varies between individual microorganisms depending on what type of microorganism they are (e.g. Bacteria, Archaea, or Eukarya), what environment they are thermally adapted to (thermophile, mesophile, or psychrophile) and whether the temperature of the environment is stable or fluctuating. However despite differences in mechanistic details, the fact remains that all cells have similar structural (e.g., membranes) and functional (e.g., macromolecular synthesis, solute transport) properties, and cold temperatures affect them in similar ways. The approach adopted in this paper is to describe the effects that cold shock has on the main biological targets of the cell, in association with the

resulting cellular responses. This is described by considering the effects of cold from the outside of the cell to the inside. In addition, cold sensing mechanisms are considered and a comparison of cold shock to other cellular stresses is included. In view of the dominance that cold environments have in terms of the Earth's biosphere, and the importance of cold active biotechnology products, it is likely that the field of cold shock and cold adaptation will expand rapidly in the coming years.

1. Introduction

The term “cold shock” refers to the exposure of an organism to a sudden decrease in temperature, and the cellular response to this is termed the “cold-shock response."Earth’s biosphere ranges in temperature from above 100 °C to below 0 °C. However, the bulk of the biosphere is cold (e.g., most of the ocean, alpine regions, and natural environments exposed to the winter season, as well as artificially refrigerated environments). As a result, it may be expected that most life forms would be capable of mounting a cellular response to the cold. In addition to relatively constant cold environments, cells may also be exposed to a sudden drop in temperature. A good example of this is the excretion of an enteric microorganism from the warm (38 °C) gastrointestinal tract of an animal and exposure to the colder surrounding environment. In this case, if the microorganism is to recolonize a host at some future stage, it must be capable of a cellular response that will permit survival, and perhaps even growth in an environment with a reduced ambient temperature.

The cold-shock response has been studied in most life forms including eukaryotes (e.g., animals and plants, yeast, fungi), bacteria, and archaea, thereby providing a cross section of physiological and genetic responses to the cold from members of all three domains of life. While this potentially provides a broad perspective, the greatest body of knowledge centers on the cold-shock response in bacteria and hence a truly comprehensive comparison is not presently feasible.

When considering how cells may have evolved to cope with cold environments, it is useful to reflect on the range of growth temperatures that permit microbial growth and colonization. Hyperthermophiles from hydrothermal vents live at temperatures above 100 °C. *Pyrolobus fumarii* survives at temperatures up to 113 °C with optimal growth at 105 °C. Due to the cellular adaptation that has taken place to enable it to grow well at such high temperatures, it is unable to grow at temperatures below 90 °C. Therefore, when *P. fumarii* (and similar microorganisms) exit the hydrothermal vent and are exposed to the surrounding cold ocean waters (1–5 °C), it is very unlikely that they would remain metabolically active, let alone able to grow. In this case, a cold-shock response may confer enhanced survival to the cold, but it could not produce a cellular response that would allow the cells to grow. In this context, it is worth noting that no microorganisms have yet been identified that will grow at temperatures spanning more than about 45 or 50 °C. In contrast to the enormous extremes a hyperthermophile may be exposed to, a mesophilic microorganism such as an enteric strain of *Escherichia coli*, which grows optimally at 37 °C, may be expected to be able to cope with a cold shock of 20 or 30 °C. Conceivably this would not only permit survival but also growth at the reduced temperature.

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At the cold end of the abiotic extreme, psychrophilic (growth temperature maximum of 20 °C or less) and psychrotolerant (growth at low temperature but with a maximum growth temperature above 20 °C) microorganisms have evolved cellular processes for growth where low temperature is the norm. Therefore, while there is a low temperature limit that even cold adapted microorganisms are unable to grow at, they are clearly genetically and physiologically geared for coping with the cold. It may therefore be expected that they would possess novel genes involved in cold adaptation, as well as constitutively maintaining the expression of genes that are otherwise only induced during cold shock in mesophilic or thermophilic bacteria. Due in part to this concept, plus the finding that some genes are transiently induced during cold shock while others remain expressed during growth at low temperature, the terms cold shock and cold acclimation are used to distinguish the two classes. The terms are context dependent as a cold acclimation gene in one organism may be a cold-shock gene in another. In general, genes that are cold-shock genes in mesophiles may be cold acclimation genes in psychrophilic or psychrotolerant microorganisms (e.g., *cspA* in *E. coli* compared with *Arthrobacter globiformis*).

The cold-shock response has been most extensively studied in the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *Bacillus subtilis*. A range of other mesophilic, thermophilic, psychrophilic, and psychrotolerant bacteria have also been studied (Table 1). A common approach has been to use two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) to generate protein profiles (proteome) to study global gene expression in response to cold shock or growth at cold temperature. In *E. coli*, two classes of proteins are synthesized in the cold-shock response. The first class are immediately synthesized while the second class are involved in reestablishing growth in the cold. The response is sequential with expression of the first class generally being a prerequisite for the second. In *E. coli* a lag in growth accompanies the cold-shock period. However, in bacteria such as *B. subtilis*, there is no lag. During the cold shock, synthesis of housekeeping proteins tends to be repressed, though this is organism-dependent. Other factors that influence the response include the magnitude of the cold shock and the composition of the growth medium. In cold adapted microorganisms there generally appears to be a lower number of cold-shock genes expressed, but the number of cold acclimation genes tends to be higher.

Microorganism	**Cold regulated target (cold shock or cold acclimation)**	**Cold shock condition (°C)[a]**
Psychrophilic and Psychrotolerant bacteria		
Acinetobacter sp. (psychrotolerant)	Esterase	
Aquaspirillum arcticum (psychrophilic)		10 to 0 (14 proteins)
Arthrobacter globiformis (psychrotolerant)	CSD[b]	25 to 4 (29 proteins)
Bacillus		20 to 0 (11 proteins)

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psychrophilus (psychrotolerant)		
Patoea ananas (ice nucleating)	Hsc25 (protein refolding)	
Pseudomonas fluorescens (ice nucleating)	26kda (antifreeze?)	18 to 4
Pseudomonas fragi (psychrotolerant)	CapA,B & TapA,B (CSD proteins)	30 to 5 (25 proteins)
Pseudomonas syringae (psychrotolerant)	*hut* operon (histidine utilisation)	
Shewanella violacea (psychrophile)	CspA,G	8 to4 or –1
Vibrio ABE-1 (psychrophile)	Isocitrate dehydrogenase	
Vibrio ANT-300 (psychrophile)		13 to0 (39 proteins)
Yersinia enterocolitica (psychrotolerant)	CspA1,A2	
Mesophilic bacteria		
Bacillus subtilis	CspB,C,D CheY (chemotaxis) Hpr (sugar uptake) S6,L7,L10,L12 (ribosomal proteins) Elongation factor (EF-G, EG-Tu, EF-Ts) Peptidyl propyl *cis/trans* isomerase (PPiB) Trigger factor (Tig) Cysteine synthase (CysK) Ketol-acid reductoisomerase (IlvC) Glyceraldehyde dehydrogenase (Gap) Triosephosphate isomerase (TIM) Glutamine synthase (GlnA) Betaisopropyl malate dehydrogenase (LeuC) Threonine synthase (ThrC) Chorismate synthase (AroF) Fructosebisphosphate aldolase (Fba) Ionsine monophosphate (GuaB) Enterochelin synthase (DHBA) Thioesterase (Srf4) Sporulation factors (SpoVG, Csi12P) Des (desaturase) Aconitase	37 to15 (37 to 53 proteins)
Cyanobacteria: *Anabaena*	RbpA1, RbpA2, RbpA3, RbpC (RNA binding protein) RNA helicase (ChrB, ChrC) Ribosomal protein (S21)	

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	Glucanotransferase (Lti2)	
Cyanobacteria: *Synechococcus*	CipB	
Cyanobacteria: *Synechocystis*	Desaturase (desA, desB, desC, desD) Chaperone (ClpB) Protease (ClpP)	
Cyanobacteria: *Spirulina platensis*	Desaturase (desA, desD)	
Escherichia coli	CspA,B,G,I (cold shock domain protein) CsdA (DEAD box RNA helicase) RbfA (ribosome binding factor) NusA (anti/termination factor) PNP (ribonuclease) RecA (recombination factor) IF-2 (initiation factor) H-NS (nucleoid associated protein) GyrA (DNA topoisomerase) Hsc66 HscB (chaperonin?) Trigger factor (chaperone) Dihydrolipoamide acetyltransferase Pyruvate dehydrogenase	37 to15 37 to4 (69 proteins)
Lactococcus lactis	CspA,B,C,D (RNA stabilisation) P170 Glycolytic activity	30 to8–10 (12 proteins)
Listeria monocytogenes	Flp (ferritin-like proteins)	
Mycobacterium smegmatis	CipMa (Hlp) (histone-like protein)	37 to10
Rhizobium leguminosarum	CSD	
Salmonella typhimurium	CspA,B	37 to24– 25
Vibrio cholerae	VicH (gene regulator)	
Vibrio vulnificus		23 to13 (40 proteins)
Thermophilic bacteria		
Bacillus caldolyticus	CSD	
Streptococcus thermophilus	CSD 21.5 kda protein	42 to20–15
Thermatoga maritima	CSD	
Yeast		

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Saccharomyces cerevisiae	TIP1, TIR1,2 (membrane associated) BFR2 (protein transport to Golgi) LOT1/FBA1 (fructose biphosphate aldolase) LOT2/RPL2B, L39 (ribosomal proteins) LOT3/NOP1, NSR1 (nucleolar protein/ribosome biogenesis) LOT5/YKL183w LOT6/YLR011w Cer1p/Lhs1p/Ssi1p (protein translocation into endoplasmic reticulum) Spb4p (RNA helicase)	30 to10
Trichosporon pullulans (psychrotolerant)		21 to5 (26 proteins)
Archaea		
Methanococcoides burtonii (psychrotolerant)	DeaD (DeaD box RNA helicase)	23 vs 4
Sulfolobus islandicus	Reverse gyrase (reduced expression)	80 to65

[a]if known, the temperatures used to induce cold shock, and the number of cold shock induced protein spots identified by 2D-PAGE.
[b]CSD (cold shock domain protein) and CspA are used interchangeably in this table and where specified (e.g., CspA), the name has been included.

Table 1. Microorganisms and genes involved in cold shock

In view of the different temperature ranges and nature of the environments (thermally stable or changing) that microorganisms colonize, it would be anticipated that a variety of mechanisms would be discovered that reflect different evolutionary processes to cope with cold shock. At present, the field is still in its infancy (particularly in comparison to studies on heat shock), although a number of important findings have been made. Some of the generally accepted principles are, that low temperature constrains cellular activity by lowering enzyme reaction and solute uptake rates, reduces membrane fluidity, stabilizes inhibitory nucleic acid structures, and forms intracellular crystalline ice. As a result, research efforts have focused on the ways in which microorganisms cope with these impositions to cellular function.

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The aim of this review is to discuss the effects of cold shock on the main biological targets of the cell, to highlight cellular responses to these effects, and to overview the different types of microorganisms which have been investigated and have provided insight into cold-shock mechanisms. A cartoon of the main cellular targets involved in the cold-shock response is shown in Figure 1. This also serves to highlight the main areas covered in the review. A detailed analysis of cold-shock regulatory mechanisms is not included in this review as there is insufficient data to infer general principles. However, a coverage of developments in this area from a number of microorganisms is presented. The field is rapidly expanding and the reader is likely to find an increasing number of articles and reviews in the forthcoming literature. The references in this review are primarily contemporary reviews and articles in generally accessible journals covering the breadth of the field.

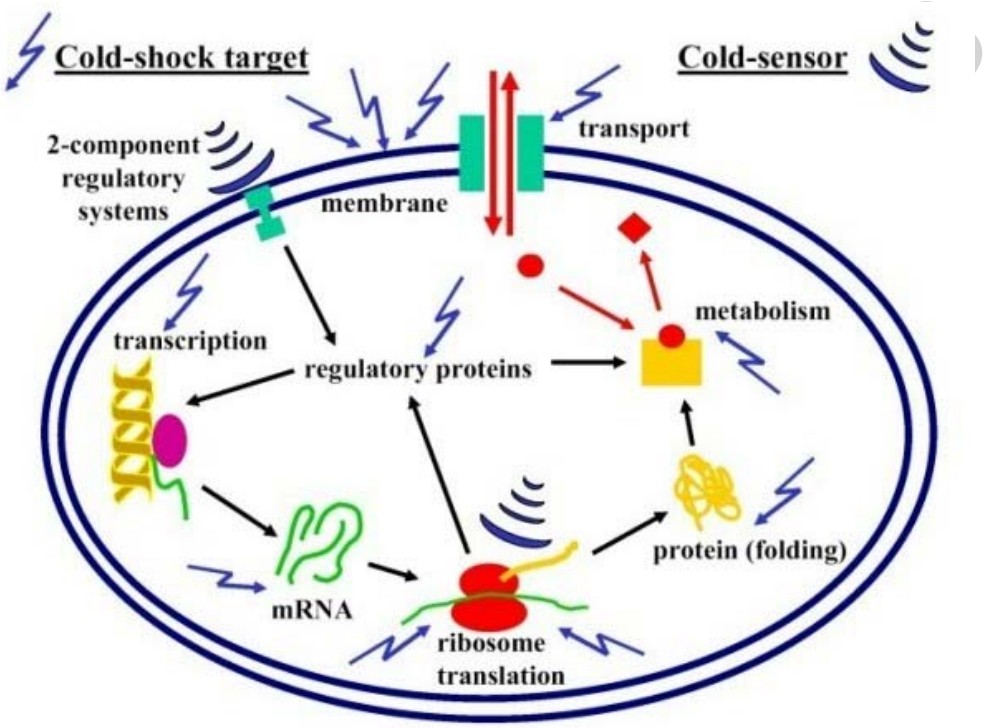

Figure 1. The effects of cold temperature on a generic microorganism
Specific biological targets are combined in the figure to illustrate cellular processes affected by cold shock. The affected targets are therefore the sites of adaptation in the cold-shock response.

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Biographical Sketches

Ricardo Cavicchioli is a Senior Lecturer at the University of New South Wales (UNSW), Sydney, Australia, since 1999. He was a Lecturer at UNSW from 1994 to 1999, a post-doctoral fellow at University of California, Los Angeles (UCLA) from 1991 to 1994. He completed his Ph.D. at the University of New England in Armidale, Australia, in 1991, and was awarded his M.Sc. from James Cook University (JCU) in Townsville, Australia in 1986, and his B.Sc. Hons. from JCU in 1984. Areas of active research included the heat shock response in the yeast, *Saccharomyces cerevisiae* (Hons and MSc), the molecular biology of a rumen, cellulose degrading anaerobe, *Fibrobacter succinogenes* (PhD), and the molecular genetics of two component regulatory systems for anaerobic, nitrate respiration in *E. coli* (postdoctorate). Since completing his postdoctoral studies, his research has focused on extremophiles; specifically, cold adaptation in archaea and low nutrient adaptation in marine oligotrophic ultramicrobacteria. Work on archaea has provided the first insights into gene regulation, protein adaptation, and the role of intracellular solutes in cold adaptation in this class of microorganisms. Research includes genome sequencing projects for the psychrophile, *Methanogenium frigidum* and the psychrotolerant strain, *Methanococcoides burtonii*. A branch of the research is orientated at commercial exploitation of cold active products from the archaea and other novel Antarctic microorganisms. Research on oligotrophic bacteria focuses on the ultramicrobacterium, *Sphingomonas alaskensis*. Through physiological studies of stress resistance, ribosome content, macromolecular synthesis rates, effects of growth rate and starvation, and proteomic analysis of global gene expression, it has become clear that the physiology of *S. alaskensis* is distinctly different to copiotrophic bacteria such as *Escherichia coli* and the marine strain, *Vibrio angustum* S14. Advances with molecular techniques, including gene transfer, expression of GFP reporters, cell sorting by flow cytometry and fluorescent in situ hybridization (FISH) analysis of gene expression have not only added powerful tools for laboratory based physiological studies but are providing the means for effective ecological studies.

Neil Saunders was awarded an Australian Research Council Postdoctoral fellowship in 2002, at The University of New South Wales (UNSW), Sydney, Australia. Prior to this he was a Vice Chancellor's Research Fellow at UNSW from 2000 to 2002, a Marie Curie Research Fellow at Vrije Universiteit in Amsterdam, The Netherlands from 1998-2000. He obtained his Ph.D. at the University of Oxford, UK in 1997, and his B. Sc. Hons. from the University of Edinburgh, UK in 1993. Areas of research included the kinetics of bacterial cytochrome *c* peroxidases (BSc), the biochemistry of bacterial nitrite reduction (PhD) and the molecular regulation of denitrification (postdoctorate). Current research includes genome sequencing and analysis of the psychrophile *Methanogenium frigidum*, biochemical characterization of cold-induced proteins from psychrotolerant *Methanococcoides burtonii*, and bioinformatic analysis of microbial genomes.

Torsten Thomas is a Senior Research Scientist at Nucleics Pty. Ltd., Sydney, Australia and an Honorary Research Fellow of the University of New South Wales (UNSW), Sydney, Australia since 2001. He obtained his Ph.D. from UNSW in 2001, and his M.Sc. from Rheinische Friedrich-Wilhelms Universitaet, Bonn, Germany in 1996. Areas of research included the biotechnological production of natural products (compatible solutes) with halophilic bacteria (MSc) and the structural, biochemical, and physiological adaptation of archaeal proteins to low temperatures (PhD). Other research projects included the regulation of cold-induced genes in low-temperature adapted archaea, structural cold-adaptation of bacterial elongation factor proteins, characterisation of marine bacteria with antifouling properties, and an initiating role in the genome sequencing of the psychrophilic archaeon, *Methanogenium frigidum*. Current research focuses on the development of molecular tools in the field of genomics and functional genomics. These include discovery and characterisation of novel enzymes, development of improved DNA-sequencing technologies and the development of high-throughput protein purification systems.

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