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REVIEW PAPER

Bacterial Cold Shock Response at the Level of DNA Transcription, Translation, and Chromosome Dynamics

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Abstract—Data available in the literature concerning the response of mesophilic bacteria to cold shock at the level of DNA transcription, translation, and chromosome dynamics, i.e., in terms of cell biology, are analyzed. Relevant molecular mechanisms and particular regulatory systems within the framework of a general cell response to cold shock are considered. It is suggested that a short-term response to cold shock is necessary for bacteria to transit to a viable but nonculturable state and/or for their physiological and genetic adaptation to psychrotrophic life. It is emphasized that cell responses to cold and heat shocks are different and that DNA dynamics (i.e., its supercoiling, multiple bending, and condensation) and the rearrangement of the protein-synthesizing apparatus of cells (including the induction of alternative translational mechanisms) may play a central role in cell response to cold shock. The role of molecular chaperones in cold shock response is presumably of less importance than it is in the case of heat shock.

Key words: stress, cold shock, bacteria, regulation.

1. INTRODUCTION

The response of bacterial cells to shocks and stresses has lately been one of the most attractive subjects to researchers, who have been studying it largely at a cellular level, i.e., within the scope of cell biology. Efforts of researchers have been primarily focused on the implementation of the genetic program of cell response to unfavorable environmental conditions, which chiefly involves the synthesis of new proteins in early periods after cell exposure to stress and the intensification of the synthesis of some proteins that are always present in cells. Attention is also given to the effect of stress on the dynamics of chromosomes and RNAs.

The range of the stresses studied is very wide and involves shifts in physicochemical parameters (as temperature, pH, and osmotic pressure); cell exposure to oxidizers, toxic compounds' and organic solvents; nutrient and energy starvation; the ingestion of cells by macrophages; cell attack by bacteriophages; etc.

The logic and methodology of cold shock studies originate from the logic and methodology formerly employed for the study of heat shock and related phenomena (osmotic, acidic, ethanolic, and other stresses), for which the involvement of molecular chaperones is typical [1–3].

The mechanism of cell response to cold shock is still poorly understood, while this problem is of great importance with regard to ecological safety and the development of ecological biotechnologies in the temperate and subpolar climatic regions. This review deals with the analysis of data available in the literature on cell response to cold shock at a cellular and molecular level with account of heat shock data. Such an analysis may be of interest from the standpoint of the role of the mechanism of cold shock response in the adaptation of mesophilic organisms to psychrotrophic and psychrophilic life and the role of this mechanism in the transition of cells to the viable but nonculturable state and anabiosis at subzero temperatures. These problems are closely related to general ecological problems. This relation is, however, very complex and needs special consideration.

2. DESCRIPTION OF THE PHENOMENON

Most of the cold shock response studies are performed with *Escherichia coli* [4–6], while data obtained for other bacteria are fragmentary and cannot be comprehensively analyzed. This fact should be accounted for when reading this review. In *E. coli*, a cold shock response is induced when the ambient temperature falls from 21–38°C (the so-called physiological temperature range) to 20°C or below. This means that *E. coli* cells present in soil and natural aquatic systems are under cold stress conditions most of the time.

In experiments with *E. coli* cells, cold shock is commonly induced by shifting the temperature from 37 to 10°C. After such a temperature downshift, cell growth is first arrested and then resumed after only about 4 h at a new rate determined by the new temperature conditions [4]. Within this 4-h time period, the synthesis of

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most proteins and nucleic acids is suppressed, whereas the fatty acid composition and the physicochemical state of cell membranes undergo considerable changes [4].

Some of these alterations are not specific and occur in response to other kinds of stresses. Moreover, it was shown that *E. coli* cells are able to grow at 10°C in a specially modified nutrient medium without changing the degree of saturation and the isomeric composition of their fatty acids. Accordingly, changes in the fatty acid composition of cells cannot be considered to be a typical cell response to cold shock. The synthesis of macromolecules can also be suppressed under the action of many factors [1–6].

Like responses to other kinds of shocks and stresses, cell response to cold shock involves the prompt synthesis of specific proteins, called cold shock proteins. Lowering the temperature, especially to 1–8°C, brings about alterations in translational processes, including the dissociation of polysomes and the accumulation of whole ribosomes and their subunits. It should be noted that cold shock suppresses protein synthesis just at the stage of initiation of DNA translation. Once the synthesis of a polypeptide chain has started, its elongation will continue irrespective of the cold shock conditions [5, 6].

3. SYNTHESIS OF COLD SHOCK PROTEINS

Analysis of the protein profile of *E. coli* cells by two-dimensional electrophoresis showed that they stop synthesizing most proteins in response to cold shock, so that, after 2 h, only 28 proteins are synthesized. One of these proteins, called CspA, is a new protein, i.e., not synthesized in cells grown under normal physiological temperatures. By the end of the lag period caused by the temperature downshift, 50 additional proteins are synthesized, all of which can also be found in cells grown at 37°C. Analysis of the synthesis rate of all 78 proteins showed that only 13 of them (some researchers believe that this number is somewhat different) are synthesized more rapidly at low temperatures. It is these 13 proteins, including the major cold shock protein CspA, that are called cold shock proteins [4–6]. Let us consider them in more detail to gain insight into the mechanism of the primary cell response to cold shock.

The Major Cold Shock Protein, CspA

This protein (formerly also known as F10.6 and CS7.4) is the only genuine cold shock protein, since it is synthesized only at low temperatures. The amount of CspA in *E. coli* cells exposed to cold shock may reach 13% of the total protein content of cells. In the course of the cold shock–induced lag period, the rate of CspA synthesis increases about 100-fold. These facts, together with the fair stability of CspA, indicate a unique physiological role of this protein [7], which may lie in the general regulation of cell response to cold shock and/or in chromosome condensation (see below).

Indeed, CspA has distinct motifs analogous to those found in single-strand DNA and RNA and some transcriptional activators of higher organisms. Jones *et al.* discovered a binding site for CspA in the regulatory region of the *E. coli* gene *gyrA*, which codes for the α subunit of DNA gyrase [8]. This site contains a motif ATTGG, which is also present in three copies in the promoter region of the *gyrA* gene. Thus, the α subunit of the DNA gyrase of *E. coli* is also a cold shock protein, whose synthesis is activated due to the presence of several sites for CspA binding. The enhanced synthesis of DNA gyrase likely compensates for a decrease in the activity of DNA gyrase at low temperatures, thereby providing the level of DNA supercoiling necessary for its efficient transcription.

Of interest is the fact that the second subunit of the *E. coli* DNA gyrase, the β subunit, is synthesized at the same low level at both temperatures, 37 and 10°C. The synthesis of this subunit at 10°C obviously does not need CspA, since there are no CspA binding sites in the regulatory region of the *cspB* gene. Jones *et al.* failed to reveal a direct contact between the transcriptional activator protein CspA and RNA polymerase [8], which suggests the existence of one more activator protein.

The CspA Family of Homologous Proteins

An analysis of the cold shock proteins of *E. coli* using amino acid sequence databases showed that CspA is a member of a family of eight or nine homologous proteins, from CspA through CspH (or CspI). It is obvious that this family resulted from the intense duplication of the *cspA* gene and related genes. The family is divided into several subfamilies, which differ in the function of their proteins. Strictly speaking, only three proteins of this family, CspA, CspB, and CspG, are cold shock proteins. The functions of CspA and the two other proteins partially overlap [9, 10].

CspA contains recognition and binding sites for not only DNA, but also RNA [10], which suggests that this protein may play the role of RNA chaperone [11]. It is possible that CspA is involved not only in the regulation of DNA gyrase, in the transcription of some proteins (e.g., HNS [12] and CspA itself), and in chromosome condensation, but also in the stabilization of various kinds of RNA at low temperatures [9, 10].

DnaA. This cold shock protein is an initiator of DNA replication on *oriC*. The concentration of DnaA in *E. coli* cells grown at low temperature is twofold higher than it is in cells grown at 37°C, indicating that it is actually a cold shock protein [13]. DnaA that is synthesized in response to cold shock is active only at low temperature. The initiation of DNA replication in both types of cells, i.e., grown at 37 and 10°C, is controlled by maintaining a constant ratio of the cell biomass to the number of initiation sites for DNA replication [13].

RecA. This cold shock protein is involved in recombination and repair events in cells [4–6].

NusA. This protein is involved in the regulation of DNA transcription (namely, the termination–antitermination processes) in cells grown under normal conditions and, presumably, at low temperatures [4–6].

HNS. This cold shock protein is regulated at the level of DNA transcription with the involvement of CspA, as is evident from the fact that the promoter region of HNS contains the corresponding recognition site. This histonelike protein has high affinity to bent DNA and likely promotes chromosome condensation at low temperatures [12].

CsdA. Under cold shock conditions, this protein is associated with ribosomes. There is a hypothesis that CsdA is necessary to derepress heat shock proteins after the termination of cold shock. A detectable heat shock response is observed even when the temperature increase is low (from 8–10 to 13°C). However, the greater the temperature increase after the cold shock, the more pronounced the heat shock response. CsdA may also be RNA helicase (an enzyme which is involved in RNA unwinding) [14].

TF. This molecular chaperone of *E. coli* is called the trigger factor. It is closely associated with the ribosomal 50S subunit, possesses prolyl isomerase activity (which may be a bottleneck in the folding of some polypeptides at low temperatures), is associated with a growing polypeptide chain on the ribosome, and binds to the GroEL chaperone (this chaperone is also synthesized at the normal physiological temperature). TF enhances the affinity of GroEL toward unfolded proteins and activates the degradation of some polypeptides. Unlike the level of most HSP (heat shock protein) chaperones, the level of TF in cells tends to increase as the cultivation temperature is lowered from 42 to 16 and even to 4°C. The synthesis of TF is accelerated in response to the temperature shift from 37 to 10°C even in the presence of chloramphenicol. Insensitivity of protein synthesis to this antibiotic is a typical feature of cold shock proteins. Accordingly, TF synthesis is an element of cell response to cold shock, while TF itself serves as an acclimation and protective factor at later stages of cell adaptation to low temperatures. The name "trigger factor" is arbitrary and does not have any meaning related to the specific role of TF in cold shock response [15].

Hsc66. This protein is another molecular chaperone involved in cell response to cold shock in *E. coli*. The expression of the *hscA* gene 3 h after the temperature shift from 37 to 10°C or in response to the addition of chloramphenicol increases about elevenfold. In a mutant with a lesion in the *hscA* gene, cold shock affected the synthesis of at least five other proteins [16]. Unlike TF, Hsc66 belongs to the group of Hsp70 molecular chaperones (i.e., the heat shock protein chaperones), which were detected until recently in cells exposed to cold shock. The *hscA* gene is transcribed together with the upstream gene *hscB*, indicating that HscB is also a cold shock protein [16].

In some organisms, homologues of heat shock proteins can be synthesized even more actively than when they are synthesized in *E. coli*. For instance, in *Leuconostoc mesenteroides*, HSP molecular chaperones are actively synthesized in response to heat, cold, and chemical stresses [17].

RbfA. This protein is bound to the ribosomal 30S subunit. At elevated concentrations, RbfA promotes cell adaptation to growth at low temperatures. When bound to the 30S subunit, RbfA enhances the capability of ribosomes for translation at low temperatures and thus initiates the whole system of cell response to cold shock. For this reason, some researchers consider RbfA to be the trigger of cold shock response [14].

IF2, IF2α, and IF2β—factors initiating DNA translation. These factors were the first detected cold shock proteins [4]. Remarkably, against the background of the suppressed synthesis of most cell proteins, the synthesis of ribosomal proteins continues even under the conditions of cold shock, implying that efficiently working ribosomes are necessary to cells growing at low temperatures [4–6]. This is in agreement with the fact that two genes encoding translation initiation factors, *infB* and *nusA*, occur in one operon.

Polynucleotide phosphorylase. The primary function of this enzyme in cell response to cold shock is to cleave various RNAs, presumably in coordination with the RNA chaperone CspA and/or other proteins with a similar function [4–6].

Enzymes of oxidative decarboxylation of pyruvate. Pyruvate (lipoamide) dehydrogenase and dihydrolipoamide acetyltransferase were also among the first detected cold shock proteins [4–6]. The role of these enzymes in cell response to cold shock has not yet been understood. Presumably, they are involved in the intensification of glycolysis and the suppression of the tricarboxylic acid cycle, i.e., in the processes that are observed upon the retardation of cell growth, the adaptation of cells to stresses, and cell transition to a nonculturable or resting state.

Desaturase of membrane lipids. This is the only enzyme of membrane lipid metabolism that is involved in cell response to cold shock. It is noteworthy that this cold shock protein is found in *Bacillus subtilis*, but not in *E. coli* [18].

γ-Glutamyltranspeptidase. This cold shock enzyme of glutathione metabolism is found in antarctic psychrotrophic bacteria [19].

4. SOME HYPOTHESES ON GENERAL REGULATORY MECHANISMS INVOLVED IN COLD SHOCK RESPONSE

DNA Dynamics

In spite of the fact that there is virtually no direct experimental evidence, the abundance of cold shock proteins capable of interacting with DNA allows some inferences to be drawn. In response to cold shock, a cell

provides for chromosome condensation by supercoiling and bending DNA. This makes the DNA more stable under unfavorable conditions and intensifies DNA transcription (which otherwise can be blocked by low temperature). The existence of the replication-initiating protein DnaA and the observation of the same dependences relating initiation frequency and the ratio of the biomass to the number of initiation sites at low and normal physiological temperatures indicate that the cell cycle is highly coordinated at low temperature and permits neither considerable variations in the cell size nor the existence of involution forms [13].

The high degree of coordination of DNA replication with other processes in cells is confirmed by the control of DNA supercoiling at the level of transcription of the α subunit of DNA gyrase [8].

Regulation at the Level of DNA Transcription

The role of such regulation in the general regulatory system of cold shock response is still a subject of debate. The discovery of motifs responsible for the binding of the major cold shock protein CspA to single-strand DNA and the detection of enhancer-like sites in the regulatory sequences of some cold shock genes led Jones *et al*. to the hypothesis that regulation at the level of transcription is an important, or even the major, mechanism of regulation of cold shock protein synthesis [8]. In the course of time, this standpoint underwent modification but did not lose its significance. In particular, it was shown that the attenuation mechanisms of transcription of the *cspA* gene provide for a transient pattern of CspA synthesis. Experiments with reporter genes (such as the *cspA* and *lacZ* genes fused in their promoter regions) showed that the activity of the *cspA* gene promoter is actually induced about ten times less than can be expected from the amount of CspA synthesized [19]. The process of cold shock gene transcription is characterized by the functioning of a unique mechanism of premature termination, which involves mRNA. The mRNA of *cspA* contains a very long 5'-terminal nontranslatable sequence (5'-NS) of 159 bp, which is able to form hairpinlike structures and functions like the *rho*-independent system of premature termination. This sequence, called cold box, is typical of all cold shock proteins. Thus, CspA negatively regulates its own transcription [20].

The problem of the regulation of the expression of cold shock genes has also an aspect related to guanosine tetraphosphate (ppGpp). This aspect will be briefly considered below.

The Regulation of Cold Shock Protein Synthesis at the Level of Translation

This type of regulation is the least studied aspect of cell response to cold shock. It has been established that the response of cells to cold shock is induced by the transient blocking of initiation of translation [21], as occurs in response to heat shock or exposure to chloramphenicol [22]. On the one hand, this blocking must promote the accumulation of mRNA, especially the mRNA of *cspA*, thereby activating the expression of this gene [22]. On the other hand, the absence of translation must block the synthesis of cold shock proteins. In *E. coli*, DNA translation at low temperatures involves three ribosomal proteins, IF2, CsdA, and RbfA, which were described above.

The predominant synthesis of cold shock proteins at low temperatures on the normally nontranslatable ribosome implies that there is an alternative mechanism for the initiation of translation. In fact, it was shown that the mRNA of cold shock proteins has the so-called downstream box located somewhat downstream of the AUG starting site of translation. This box is responsible for the binding of the corresponding mRNA to the ribosome, thereby implementing the alternative mechanism of initiation of translation [21].

There are at least two other factors contributing to the alternative translation, namely, the enhanced stability of the mRNA of cold shock proteins at low temperatures and the stability of the cold shock proteins themselves [23, 24].

Both these properties are due to the chaperone function of cold shock proteins, primarily CspA. Like an RNA chaperone, CspA prevents the formation of unwanted secondary structures in cold shock mRNAs, thereby providing for their efficient translation at low temperatures. At the same time, CspA promotes the cleavage of these mRNAs at normal physiological temperatures [11].

Dynamics of Membrane Lipids

Change in the lipid composition of cytoplasmic membranes is a universal response of cells to temperature shifts. These membranes occur either in the liquid-crystalline state under normal conditions or in the gel-phase state at low temperatures. Many organisms compensate for the transition of their membranes to the gel-phase state by changing the degree of saturation of membrane phospholipids (a more unsaturated phospholipid is characterized by a lower melting point and a higher degree of fluidity than a less unsaturated phospholipid). This kind of cell response to cold shock, known as homeoviscous adaptation, was found not only in *E. coli*, but also in many other microorganisms (cited from [21]).

The enzymes involved in this kind of cell response are desaturases (under aerobic conditions) and 2,3-dehydrases (under anaerobic conditions). In *E. coli* and many other bacteria, these enzymes are responsible for the synthesis of unsaturated fatty acids (as *cis*-vaccenic acid). The enhanced synthesis of such fatty acids at low temperatures is favorable to the cytoplasmic membrane. It should be noted that cold shock does not induce the de novo synthesis of saturases and 2,3-dehy-

drases in *E. coli* cells, but only enhances their synthesis. Strictly speaking, these enzymes are not cold shock proteins in *E. coli*, although the desaturase of *B. subtilis* is a genuine cold shock protein [18].

It is possible that the significance of the proportion between saturated and unsaturated fatty acids in cell response to cold shock is overestimated. For instance, Klein *et al.* showed that the key part in the response of *B. subtilis* cells to cold shock is played by the conversion of *iso* branched fatty acids into their *anteiso* forms (primarily, *anteiso*-C15:0 and *anteiso*-C17:0 acids) [25].

There are some other hypotheses on the modulation mechanisms of membrane fluidity, such as the phosphorylation–dephosphorylation of membrane proteins [19]. These hypotheses, however, have not yet received a strong experimental underpinning.

It would be reasonable to suggest that downward temperature shifts must affect the structure and composition of outer bacterial membranes. Indeed, Carty *et al.* showed that *E. coli* cells respond to cold shock by incorporating palmitoleic acid into lipid A and drastically decreasing the content of lauric acid in this lipid [26].

5. INTEGRATION AND OVERLAPPING OF PARTICULAR GLOBAL REGULATORY SYSTEMS IN CELL RESPONSE TO COLD SHOCK

Interaction with the System of Stringent Control

The blocking of normal translation in response to cold shock and the absence of the conventional site of initiation of DNA translation make the *relA*-dependent guanosine tetraphosphate synthetase (the so-called stringent factor), which is bound to site A of the ribosome, nonfunctional, irrespective of whether or not amino acid–laden tRNAs are present. As a result, cells exposed to cold shock behave in a way opposite to that observed under nutrient deficiency (in other words, cells behave similarly at low and high ppGpp concentrations). This implies that cells with blocked translation not only fail to use RNA and proteins (primarily, ribosomal) to compensate for nutrient deficiency, as under the stringent ppGpp control, but even continue to synthesize RNA and cold shock proteins, which is an element of cold shock response [27].

Interaction with Chaperones

It is reasonable to suggest that cells exposed to cold shock require chaperones, since cold shock must affect the secondary structure of existing and newly synthesized proteins. Some relevant data were discussed above. Here, it should be mentioned that Chow and Tung showed that the enhanced expression of the heat shock genes *dnaK/dnaJ* and *groEL* confers freeze tolerance to *E. coli* cells [28]. Paradoxically, cells must endure heat shock to be able to tolerate cold shock. Strictly speaking, there is no direct effect of heat shock on the cold shock response; rather, we are dealing with the effect of short-term (2–5 h) exposure to low temperature on the remote adaptation of bacteria to unfavorable conditions. The proteins that are involved in this process are called cold acclimation proteins [29, 30].

Relationship between Cold Shock Response and Stationary-Phase Regulation

On this point, it is worth mentioning the work of Graumann and Marahiel [31] in which they identified the cold shock proteins CspB and CspC as the major proteins of stationary-phase *B. subtilis* cells.

6. SOME GENERAL REMARKS

Admitting that the problem of cold shock response at the cellular level is far from being properly understood, I will still try to approach this topic, concentrating on two possible aspects of analysis of the data presented above: first, the role of cold shock response in bacterial survival of unfavorable conditions and/or bacterial adaptation to psychrotrophic life and, second, the analogy between heat shock and cold shock responses.

It should be noted that the role of the physiological response of cold shock–exposed bacteria in their short-term survival of unfavorable conditions (in the given case, low temperatures) is relatively clear and lies in a rapid modification of cell metabolism. As for the role of such physiological response in long-term bacterial survival of unfavorable conditions and/or bacterial adaptation to psychrotrophic life, it is far from being well understood and falls outside the scope of this review. However, some general speculations on this problem can be presented.

It should be emphasized that the bacterial genome is one of the two major targets for modification in response to cold shock, both in relation to its stability (the supercoiling and multiple bending of DNA and, in the final analysis, chromosome condensation with the possible involvement of histonelike proteins) and in relation to its adequate expression (because the supercoiling and bending of DNA serve as regulators of genome transcription). This may be interpreted in such a way that a prompt response to cold shock is aimed at implementation and then maintenance of a state of cryptobiosis, during which the chromosome is able to slowly replicate and cells retain their ability to divide (see above). This opens the way for further physiological and genetic adaptation to psychrotrophic and even psychrophilic life. These speculations are in agreement with the fact that the cold shock response involves an alternative mechanism of DNA translation. It is obvious that the problem of the adaptive or evolutionary formation of genuine psychrotrophs, which are able to grow at temperatures close to 0°C, needs further investigation.

At the same time, the cold shock response may represent an intermediate stage during the transition of bacteria to the viable but nonculturable state. This possibility seems to be more probable for gram-negative bacteria. As was emphasized in my previous review [32], it is temperature shifts downward, usually in combination with other factors, that trigger the transition of many bacteria to the viable but nonculturable state.

The comparison between heat shock and cold shock responses shows that cell response to heat shock primarily involves the stabilization of normal translation and the synthesis of fully functional proteins with the aid of multiple molecular chaperones. Another element of the heat shock response is a modification of the transcriptional apparatus of cells, which manifests itself in the functioning of RNA polymerase with the alternative γ subunit RpoH. The role of molecular chaperones in the cold shock response is less important than it is in the heat shock response. This fact becomes more understandable if one takes into account that proteins synthesized and functioning in cells grown at normal physiological temperatures are denatured more easily at elevated than at decreased temperatures.

Thus, cell responses to heat and cold shocks differ in the strategy of metabolic rearrangement, depending on the physicochemical peculiarities of the processes occurring at the level of cellular macromolecules (as proteins and nucleic acids).

In nature, the physiological response of cells to cold shock, followed by periods of their adaptation to harsh environmental conditions and evolution to extremophily, is typical of, for instance, archaea [33]. The mechanisms of bacterial adaptation to low temperatures are not limited to those found in *E. coli*. Thus, the cold shock response of *Mycobacterium tuberculosis* involves the differential expression of sigma-factor genes [34], as occurs in response to heat shock in enterobacteria. It is beyond doubt that further investigations of cell responses to other shocks and stresses (desiccation, exposure to toxic compounds and nonaqueous phases, etc.) using a wider range of microorganisms should significantly contribute to our understanding of this phenomenon.

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