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Purnima Bhargava is in the Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

Cold shock proteins from microorganisms

M. K. Chattopadhyay

It is well known that microorganisms, like the higher eukaryotes, respond to sudden change of temperature by synthesizing a new group of proteins. Literature is enriched with reports on the heat shock proteins (HSPs) of microorganisms. The cold shock proteins (CSPs) from microorganisms, on the other hand have started coming to the limelight only recently.

The chapter was opened in 1987. P. G. Jones *et al.*[1] from the University of Michigan, reported that when growing cells of *Escherichia coli* were shifted from 37° to 10°C, growth was halted for several hours and then started with a new rate. During this renewed growth, about two dozens of new proteins were found to be synthesized. These proteins were essential for transcription and translation[1].

Subsequently it was shown that the major CSP in *E. coli* was a cytoplasmic protein (mol. wt 7.4 kDa) having DNA-binding properties. It was named CS 7.4 and the gene encoding it was designated *cspA*. The protein was found to be autoregulated. From several observations it was hypothesized that CS 7.4 in *E. coli* might be an antifreeze protein[2]. Subsequent studies, however, did not validate this idea but striking sequence similarity and to some extent sequence identity were found between CS 7.4 and a family of eukaryotic DNA-binding proteins, called Y-box transcription factors[3]. Further, it was reported that CS 7.4 could enhance the rate of transcription of the genes encoding H-NS[4] and the A subunit of DNA gyrase[5]. The synthesis of both of these proteins was already known to be sustained in *E. coli* following cold shock. Proteins like the transcription factors influence the rate of transcription by binding to the promoter region of the gene. When the promoter of several cold shock genes of *E. coli* were cloned and sequenced a CCAAT motif was found to be conserved[6]. The same motif was known to be involved in the binding of CS 7.4 to the promoter of the gene which codes for H-NS. Similarly an ATTGG motif, which is known to be essential for binding of Y-box transcription factors to their target DNA, and also known to be involved in the binding of CS 7.4 to the promoter of *gyrA* (gene responsible for the synthesis of DNA, gyrase A in *E. coli*) was found to be present in several cold shock promoters of the same organism including the promoter of *cspA*[5]. It was shown by Jones and her co-workers that activation of *cspA* expression upon cold shock preceded that of other cold shock genes[1]. Hence it has been suggested that CS 7.4 is a transcriptional enhancer for several of the CSPs in *E. coli*.

A CSP of 7.36 kDa was purified from *Bacillus subtilis*. This protein called Csp B, was found to have a sequence similarity with CS 7.4. The viability of the cells from a mutant strain, incapable of synthesizing Csp B, was drastically reduced at –80°C. This effect could be partially minimized when the cells were incubated at 10°C prior to shifting to –80°C (ref. 7). In this case also an antifreeze role was postulated for Csp B. The protein was found to have a 40% sequence identity with the nucleic acid binding domain of Y-box factors. This motif which is called the cold shock domain (CSD) is known to be conserved from bacteria to humans. The three-dimensional structure of Csp B, elucidated recently by X-ray crystallography[8] and nuclear magnetic resonance spectroscopy[9] revealed the presence of an antiparallel five stranded *a*-barrel with strands connected by turns and loops. The structure bears resemblance with the structure of *Staphylococcal* nuclease and gene-5 single stranded DNA-binding protein.

Synthesis of CSPs in *E. coli* had been reported to be induced by treatment of the cells with erythromycin, chloramphenicol, fusidic acid, spiramcyin and tetracyclin. Aminoglycosidic antibiotics were earlier reported to induce the HSPs in *E. coli*. All these antibiotics are known to act on ribosomes. Hence a putative role

of *E. coli* ribosomes, as a sensor of heat and cold shock network, was suggested[10].

Stringent response is an austerity measure adopted by the cell. When there is a deficiency of amino acids in the culture medium, protein synthesis in *E. coli* gets discontinued. In this condition synthesis of ribosomal RNA (rRNA) is also stopped. During the starvation of amino acids two unusual nucleotides, viz. guanosine-5′-triphosphate-3′-diphosphate (pppGppp) and guanosine-5′-diphosphate-3′-diphosphate (ppGpp) accumulate within the cell. These two nucleotides are believed to turn off rRNA synthesis to ensure that the ribosomes will be made only when sufficient amino acids are available for protein synthesis. Recently, the effect of stringent response on the induction of CSPs in *E. coli* was studied. Wild type cells, growing at 37°C in presence of all the 20 amino acids, were filtered and suspended for 10 minutes in a medium with methionine as the only amino acid. Subsequently when the cells were cold-shocked at 10°C and all the amino acids were supplemented to the minimal media, the synthesis of several CSPs including that of CS 7.4 was found to be severely inhibited. However synthesis of H-NS was not appreciably affected and synthesis of another CSP was positively regulated by increase in the intracellular level of pppGpp and ppGpp. An overproduction of these two nucleotides caused by the nutritional downshift, resulted in increased synthesis of two major HSPs in *E. coli*. DnaK and GroEL (ref. 11).

A protein that served the dual role of a HSP and a CSP was found in the baker's yeast *Saccharomyces cerevisiae*[12]. Similarly a gene encoding a membrane protein in the cellular slime mold *Dictyostelium discoideum* was reported to be induced upon heat shock, cold shock and cadmium[13].

What can be the exact function of CSPs in microorganisms? As shown in Table 1 in *E. coli* they may be required to sustain gene expression and protein synthesis in host cells at lower temperature. A recently published report reveals that there is a family of proteins in *E. coli* comprising of Csp A (CS 7.4), Csp B, Csp C and Csp D. The genes encoding them are mapped at 79, 35, 40 and 19 minutes respectively of the *E. coli* chromosome. Csp B, Csp C and Csp D bear 79%, 70% and 45% identities respectively to Csp A. Among the four genes, so far *cspA* and *cspB* have been known to be cold shock inducible[14]. In another investigation the homologue of *cspA* has been found to exist in the genomes of some psychrotrophic strains of *Arthrobacter*, *Micrococcus* and *Pseudomonas*, isolated from the soil of the Schirmacher oasis of Antarctica. The homologue was expressed in *Arthrobacter* and *Pseudomonas* both at 4°C and 22°C but a 10-fold increase in transcription was noticed when the temperature was decreased from 22°C to 4°C. The time required for maximum induction of the gene *in vivo* (1 h) was the same with the time taken for the reinitiation of growth of the organism after cold shock. Hence it was indicated that the homologue of *cspA* might be required for the growth of the Antarctic psychrotrophs both at 4° and 22°C but the requirement might be increased at lower temperature[15]. If a definite role of CSPs behind the cold adaptation of microorganisms can be established, by genetic manipulation it may be possible to create a recombinant strain which will be helpful for scavenging the huge amount of organic wastes from the Mt Everest, Antarctica or Siachin glacier. CSPs in microorganisms, therefore, offer a new area deserving intensive investigation and massive exploration.

Table 1. Function of some cold shock proteins in *E. coli*

Protein	Function
CS 7.4 (CspA)	Exact function not known, putative transcriptional enhancer for other cold shock genes in *E coli*
Nus A	Termination of transcription.
H-NS	Histone-like protein, believed to help compaction of DNA in *E. coli*.
RecA	General recombination, repair of DNA, induction of lambda phage.
Subunit A of DNA gyrase	Maintains supercoiling of DNA.
Polynucleotide phosphorylase	Believed to catalyse the degradation of mRNA.
Factors 2α and 2β	Initiation of translation.
Lipoamide dehydrogenase Lipoate acetyltransferase	Two of the three enzymes in pyruvate dehydrogenase complex which catalyses oxidative decarboxylation of pyruvate to acetyl CoA.

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M. K. Chattopadhyay is in the Centre for Cellular and Molecular Biology, Hyderabad 500 007, India