Acta Biochimica Polonica

Vol. 41 No. 4/1994

QUARTERLY

Minireview

The cold shock response in microorganisms

Krystyna I. Wolska

Institute of Microbiology, University of Warsaw, Nowy Świat 67, 00–046 Warsaw, Poland

Received: 28 August, 1994

Key words: cold shock, adaptive response

Balanced growth of every microorganism is maintained within a well-defined temperature range. Within the normal range of temperature, the cell composition is very similar and any minor upshift of temperature results in the instantaneous growth at the characteristic growth rate. Temperature shifts outside of that range cause pronounced physiological changes, which are essential to support growth at high and low temperatures. It is believed that these changes serve adaptive functions.

Up-to-date, the best characterized adaptive response is the heat shock response, universal for prokaryotic and eukaryotic cells. The functions of heat shock proteins [1–3] and the molecular basis of regulation of the heat shock response [4–6] are described in detail.

Recent extensive studies on the physiology of the cell at low temperature, especially but not exclusively *Escherichia coli*, lead to the discovery of the cold shock response. Transferring *E. coli* cells from 37°C to 10°C results in a 4 h lag period of growth followed by reasumption of growth with the generation time of 24 h [7]. The growth resumption is observed only when the temperature does not drop below 7.8°C [8]. Several experimental data suggest that the inhibition of translation initiation is responsible for the lag period and the minimal growth temperature [7, 9–11]. During the growth lag the number of synthesized proteins is dramatically reduced and at one time-point only about two dozen proteins are made [7].

The cold shock response includes specific changes in gene expression following the temperature downshift and is characterized by: a) transient induction of several cold shock proteins despite the severe reduction of total protein synthesis, b) continuous synthesis of transcriptional and translational proteins during the lag period and c) specific repression of heat shock proteins [7, 12]. After the transfer of *E. coli* to 10°C the response and therefore the adaptive changes of cell physiology, which allow the subsequent growth resumption, reach their maximun during the 4 h lag period. The cold shock response is induced with any downshift of at least 13°C and its magnitude depends on the range of temperature shift [8].

A lot of information about the cold shock response have been obtained during the last few years. Several cold shock proteins were identified [7, 13–21]. Moreover, the possible regulators of cold shock proteins expression were discovered recently [22–24] and also the interaction of the cold shock response with the stringent response [12] and the induction of the response by other stimuli [25, 26] were revealed.

COLD SHOCK PROTEINS

Escherichia coli

Following a shift to 10°C, 13 cold shock proteins are expressed at the rates 2 to 200 times

greater than at 37°C, as was identified by two-dimensional gel electrophoresis followed by autoradiography of total [^{35}S]methionine labelled cell extracts. Some of these proteins, listed in Table 1, were identified and were shown to be involved in various crucial cellular processes [7, 8, 16].

Protein NusA has a dual cellular function. It can act as transcription termination factor but, in a specific situation created by N-antitermination complex, its opposite antitermination activity is revealed (for review see [27, 28]).

Initiation factor 2 (IF2) mediates the binding of charged tRNA^{fmet} to the 30S ribosomal subunit for initiation of translation. In addition, IF2 helps the association of the two ribosomal subunits and has a GTPase activity in the presence of ribosomes (for review see [29, 30]). The genes encoding two proteins, the members of *nusA-infB* operon [29, 31] were located adjacent to S15 operon which comprises *pnp* gene encoding polynucleotide phosphorylase [32] involved in degradation of mRNA [33]. Although cotranscription of the operons has been detected in some conditions, it remains to be elucidated whether *pnp* is cotranscribed with *nusA* and *infB* following the cold shock [8].

RecA protein plays a very important role in homologous recombination and SOS response (for review see [34]).

H-NS is a neutral protein with a strong DNA-binding affinity. However H-NS does not wrap DNA *in vitro*, protein binding compacts DNA significantly. Moreover, H-NS may make DNA inaccessible to other DNA binding proteins, as was suggested by its negative effect on transcription and illegitimate recombination (for review see [35]).

Yet another cold shock protein is gyrase A (α subunit of the topoisomerase DNA gyrase), the enzyme involved in the proper DNA supercoiling [36].

Other cold shock proteins (not included in Table 1) are: pyruvate dehydrogenase (lipoamide), dihydrolipoamide acetyltransferase of pyruvate dehydrogenase [16, 22] and uncharacterized proteins F84.0, G41.2, G55.0 and G74.0 [8].

Last but not least, the list of cold shock proteins should be supplemented by the CspA family.

Protein CspA (CS7.4 or F10.6) is induced 200-fold following the shift from 37°C to 10°C and then it contributes to 13% of total protein synthesis. Therefore CspA has been named the major cold shock protein [7, 16]. Subsequently it has been proved that CspA is induced at the transcriptional level [25, 37]. Since the induction of CspA occurs immediately after the temperature shift, *cspA* gene expression may be repressed at high temperature by a specific repressor, which becomes inactive at low temperature [8]. Unlike other cold shock proteins, even traces of CspA are not detected at high

Table 1

Cold shock proteins in E. coli.

IF2, initiation factor 2; PNPase, polynucleotide phosphorylase; n.d., not determined.

Protein	Gene	Map position	Function
NusA	*nusA*	69′	Termination and antitermination of transcription
IF2	*infB*	69′	Initiation of translation
PNPase	*pnp*	69′	Degradation of mRNA
H-NS	*hns* (*osmZ, bglY*)	27′	DNA compactness, inhibition of transcription and illegitimate recombination
Gyrase A	*gyrA*	48′	DNA supercoiling
CspA	*cspA*	79′	DNA (RNA?) binding Activation of cold shock genes transcription
CspB	*cspB*	35′	n.d.
CspC	*cspC*	40′	n.d.
CspD	*cspD*	19′	n.d.
CspE	*cspE*	n.d.	n.d.

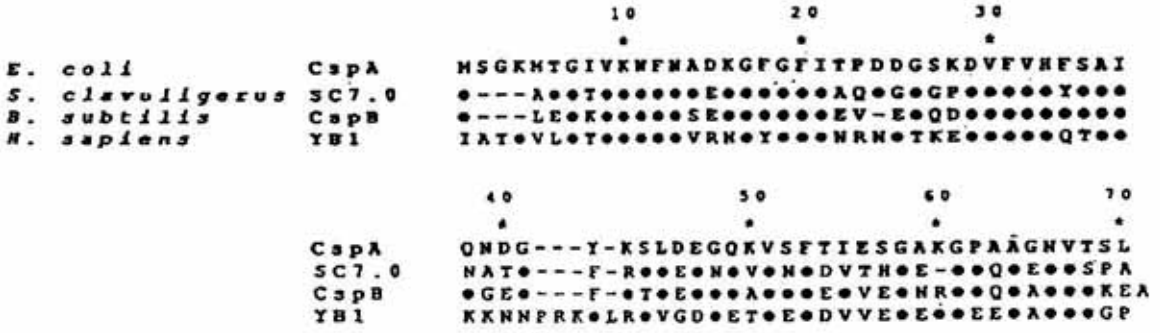

Fig. 1. Comparison of CspA-like proteins.
The sequences of *E. coli* CspA, *B. subtilis* CspB, *S. clavuligerus* SC7.0 and *Homo sapiens* "cold-shock domain" of YB1 protein are after references 16, 21, 14 and 38, respectively.

temperature due to the instability of the transcript [16]. The transcription start site (+1) of *cspA* gene was identified. *In vivo* footprinting experiment demonstrated that the region upstream of the *cspA* promoter, from bases –35 to –73, was protected, and gel mobility shift analysis showed that a cold-shocked cell extract contained a factor(s) specifically bound to the fragment containing the sequence between bases –63 and –92 [37].

CspA is a hydrophilic protein of 70 amino-acid residues encoded by the *cspA* gene mapped at 79 min [16] and shows sequence similarity to cold shock proteins from other bacteria [14, 21] and to the "cold-shock domain" of eukaryotic Y-box transcription factors [38] (Fig. 1).

Experimental data suggest the involvement of CspA in the transcriptional activation of other cold shock genes. Using *hns-cat* transcriptional fusion, it was demonstrated that CspA binds to, and stimulates transcription of the cold shock gene *hns* [23]. The positive effect on transcription of *gyrA* gene was also proved, moreover it was shown that CspA binds specifically to ATTGG sequence located in the *gyrA* promoter. It is highly probable that CspA enhances the expression of two other cold shock proteins G55.0 and G41.2 [22].

The secondary structure of CspA was analyzed by examining circular dichroism at both far- and near-UV regions; the results suggested that the protein is largely β-sheet in conformation. The predominance of β-sheet structure was confirmed further by using Fourier-transform infrared spectroscopy. The folded compact conformation was also verified by fluorescence emission spectroscopy. The protein is relatively small and contains no disulfite bonds, it is also stable to heat denaturation [39].

The tertiary structure of CspA has been determined recently by X-ray crystallography analysis [40] and nuclear magnetic resonance [41]. CspA is composed of five antiparallel β-strands forming a closed five-stranded β-barrel (Fig. 2). The surface of CspA containing an unusual

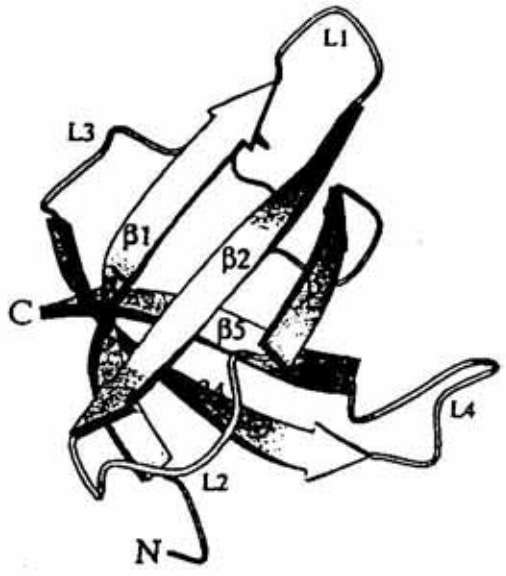

Fig. 2. Three-dimensional structure of CspA.
β-Strands are given as curved arrows and are numbered β1–β5. Loops between strands are numbered L1–L4 (after [40]).

cluster of aromatic side chains is characteristic for a protein interacting with single-stranded nucleic acids [40].

CspA can serve a general function, interacting with DNA or possibly RNA. This hypothesis is highly probable, considering the huge intracellular protein concentration (a few hundred thousand molecules per cell). The role of protein in transcription activation was already mentioned above. Its function can be executed by converting the closed complex to an open one during transcription initiation. Although it has been demonstrated that CspA recognizes a specific DNA sequence [22], it may recognize RNA as well. It can be speculated that CspA displays "RNA chaperone-like" function and acts by unwinding tightly folded RNA molecules, especially at low temperatures. "RNA chaperone--like" functions have been reported for eukaryotic RNA-binding proteins, some of which show sequence similarity to Csp [42].

E. coli has at least 4 other proteins, members of CspA family, i.e. CspB, CspC, CspD and CspE with 79%, 70%, 45% and 70% sequence identity to CspA, respectively. The cold shock inducible activation of transcription of *cspB* gene was proved recently [17].

Other bacteria

The effect of low temperature on gene expression has been examined in various bacteria besides *E. coli*.

In *Bacillus subtilis* cold-shock protein CspB with 61% identity with CspA has been found [21]. The expression of *cspB* gene is induced six- to eightfold upon temperature downshift; regulation seems to be at transcriptional level. CspB does not seem essential for bacterial growth at high and even lower but still moderate temperatures but the viability of *cspB* mutant at freezing temperatures was strongly affected [21]. The protein was crystalyzed and its structure was determined from two crystal forms. In both CspB is present as an antiparallel five-stranded β-barrel with strands connected by turns and loops. Such structure resembles that of staphylococcal nuclease and bacteriophage fd (M13) gene-5 protein. According to gel shift experiments, CspB can bind to ssDNA containing CCAAT motif. Protein surface rich in aromatic and basic residues is probably involved in nucleic acids binding [43, 44].

Another gram positive bacterium, *Streptomyces clavuligerus*, also contains a 7.0 kDa protein that closely resembles *E. coli* CspA. The two proteins share 56% sequence identity and more than 80% sequence similarity, which suggests that they may have a common biological function [14].

Cold shock response has been intensively studied in psychrophilic bacteria. Cold-adapted arctic rhizobia produced at least five proteins (52.0, 38.0, 23.4, 22.7 and 11.1 kDa) under the cold shock conditions. However, adaptation to cold by these bacteria does not seem to provide them with better survival at freezing temperature comparing with the temperate strains [15]. The synthesis of cold shock proteins (csps) and cold acclimation proteins (caps) in response to continuous growth at low temperature in the psychrophile *Aquaspirillum arcticum* was investigated. Cold shock treatments (10°C to 0°C, 5°C to 0°C, 10°C to 5°C) induced a total of 14 csps, 6 of which were induced by all three kinds of cold shocks. The production of caps in response to continuous growth at 0°C was also showed. Five out of eight caps produced were also csps which suggests that these proteins may share a common involvement in cold adaptation [20]. The presence of cold shock proteins has been reported in other psychrophilic bacteria — *Arthrobacter globiformis* S155 [19] and *Vibrio* sp. strain ANT-300 [45]. In *Vibrio* dramatic increases in the rates of synthesis of 39 proteins were induced immediately upon a shift from 13°C to 0°C [13].

REPRESSION OF HEAT SHOCK RESPONSE AND CONTINUED SYNTHESIS OF TRANSCRIPTIONAL AND TRANSLATIONAL PROTEINS

A drop in temperature (13°C or more) leads to a severe specific repression of heat shock proteins. The repression is transient and canceled gradually to a new steady state level in 60–80 min [46]. Other conditions that cause both, a decrease in the translational capacity of the cell and a stimulation of ribosome formation, such as deprivation of a streptomycin-dependent mutant of streptomycin, tetracycline addition to a partially tetracycline-resistant strain, addition of chloramphenicol, tetracycline, erythromycin or spiramycin to sensitive

E. coli strain and nutritional shift up, all result in the repression of heat shock response with the simultaneous induction of cold shock protein synthesis [26].

During the lag period, which occurs following temperature downshift, continued synthesis of many components of transcriptional and translational machinery takes place [12]. This observation is very interesting, while considering repression of transcription and translation usually observed when growth is arrested.

The search for the intracellular factor(s) which can influence the observed high level of transcriptional and translational components, pointed to the role of guanosine 5'-triphosphate-3'-diphosphate and guanosine 5'-diphosphate-3'-diphoshate, collectively abbreviated (p)ppGpp (magic spots). Variations in (p)ppGpp level do occur in response to temperature stress. In *E. coli* a downshift in temperature results in a decrease in (p)ppGpp level while an upshift has an opposite effect. The magnitude of (p)ppGpp decrease is proportional to the range of temperature downshift [47–49]. So, an inverse correlation exists between (p)ppGpp level and the synthesis of translational and transcriptional proteins (along with the induction of cold shock proteins synthesis). Neidhardt and coworkers [12] proved that increasing the (p)ppGpp level prior to temperature downshift by overproducing the enzymes that catalyse their synthesis results in decreased cold shock response. In contrast, the cold shock response in *relAspoT* mutant defected in magic spot synthesis is much stronger, moreover transfer of this mutant to 10°C results in growth without the customary lag period. The inverse correlation between (p)ppGpp content and the total rate of RNA synthesis does also exist (for review see [17]).

The change in (p)ppGpp content, which follows the temperature shock correlates well with the change observed after nutrient stress. The cold shock response mimics the nutritional shift up, whereas, heat shock response imitates the nutritional shift down. The adjustment of cell metabolism to nutritional stress was extensively studied as an example of cellular adaptive response (stringent response). Taking advantage from the analogy of the response to nutrient upshift and temperature downshift, it can be suggested that both stresses result in a physiological state where the translational capacity of the cell is insufficient relative to the supply of charged tRNA, triggering the decrease in the (p)ppGpp level with the corresponding changes in gene expression [8].

As mentioned earlier, not only a downshift in temperature but also certain inhibitors of translation (e.g. chloramphenicol, tetracycline, erythromycin, fusidic acid and spiramycin) result in the induction of cold shock response. This observation led to the proposal that the state of ribosome is the physiological sensor for the induction. It seems possible that the cold shock response is induced when the ribosomes are slowed down or made hyperaccurate. The antibiotics, which induce cold shock response, prolong the occupancy of ribosomal A site by aminoacyl-tRNA (chloramphenicol, erythromycin, spiramycin) or block it (fusidic acid, tetracycline). It is not obvious whether the physiological state of ribosome or the nature of some ribosome product is involved as a signal linking the environmental stimulus (temperature) and the cold shock response. This adaptive response, in turn, functions to correct a temperature-imposed dysfunction of translation [26].

The possible model for the cold shock regulatory network is summarily presented in Fig. 3. The details of the sensing and signalling mechanisms are still unknown.

CONCLUDING REMARKS

The extensive studies on the physiology of *E. coli* and other organisms following an abrupt shift to low temperatures already provided a lot of information on cells' response and adaptation to cold stress.

The most interesting feature which makes the cold shock response such an interesting field of study is its universality. Beside the bacteria, cold shock response and cold shock proteins were discovered and characterized in other organisms. Induction of protein synthesis in response to cold shock (21°C to 5°C) in the psychrotrophic yeast *Trichosporum pullulans* concerns 26 cold shock proteins at the maximum induction time of 12 h [50]. In *Saccharomyces cerevisiae* four genes have been identified, whose expression increased after a shift from

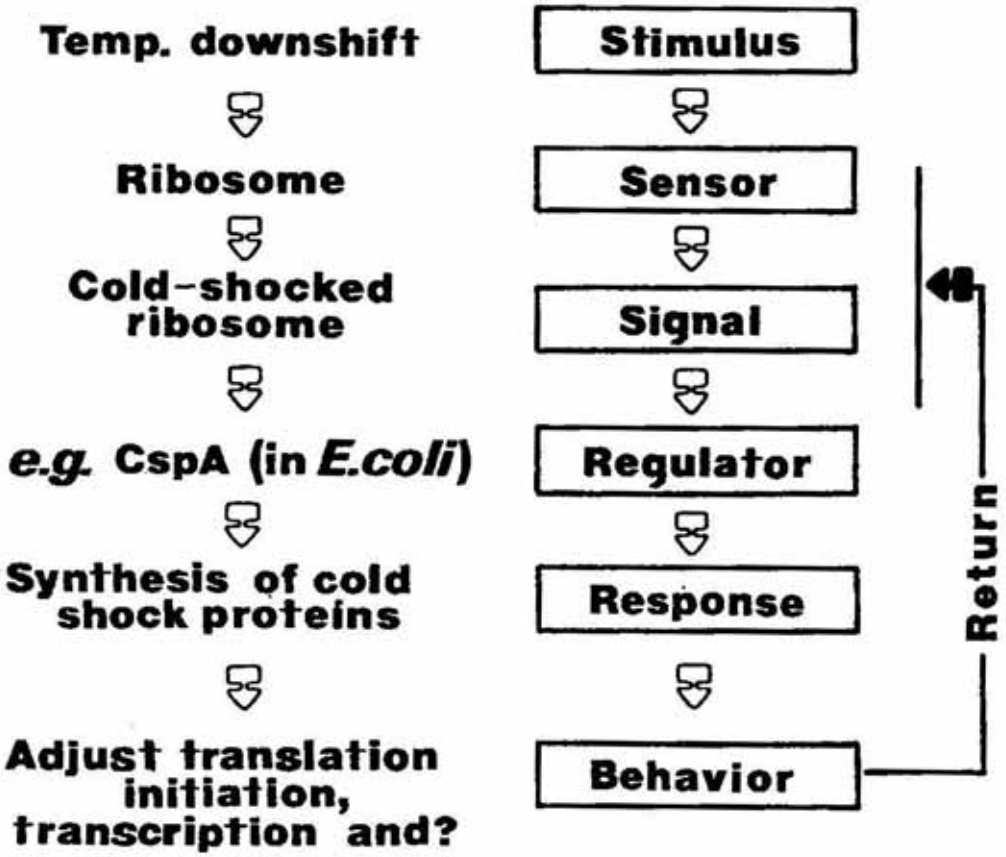

Fig. 3. Model for the cold shock regulatory network.
On the right is depicted a generic diagram of the cellular mechanism for responding to a stimulus (environmental change). The sensor is the cellular process or macromolecule that initially senses the change. It then produces signal that is transmitted to regulator, causing the latter to elicit a response that helps the cells to adapt to the stimulus. The return arrow indicates the feedback mechanism to shut down the response once the appropriate behaviour is achieved (after [26]).

30°C to 10°C. One of them, called TIP1, is a putative membrane-bound protein containing tandemly repeated serine-rich sequences. Their function is unknown yet [51]. Another nucleolin-like protein, NSR1, plays an important role in ribosomes biogenesis [52, 53]. In amoeba *Dictyostelium discoideum* cold shock induces the synthesis of a putative membrane protein and ubiquitin [18, 54] while in *Chlorella vulgaris* the accumulation of photosynthetic products was observed [55].

Many important problems remain yet to be answered. They deal with the relationships between cold shock and heat shock responses and, first of all, with working out the cold shock regulatory network. The latter will allow to answer the question why the inhibition of ribosomal function results in the induction of cold shock response. It can be hoped that the intensive researches going on in many laboratories will solve these problems in the near future.

REFERENCES

1. Ang, D., Liberek, K., Skowyra, D., Żylicz, M. & Georgopoulos, C. (1991) Biological role and regulation of the universally conserved heat shock proteins. *J. Biol. Chem.* **266**, 24233–24236.
2. Lindquist, S. & Craig, E.A. (1988) The heat--shock proteins. *Annu. Rev. Genet.* **22**, 631–677.
3. Sorger, P.K. (1991) Heat shock factor and heat shock response. *Cell* **65**, 363–366.
4. Bukau, B. (1993) Regulation of the *Escherichia coli* heat-shock response. *Mol. Microbiol.* **9**, 671–680.
5. Grossman, A.D., Straus, D.B., Walter, W.A. & Gross, C.A. (1987) σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. *Genes Devel.* **1**, 179–184.

6. Morimoto, R.I., Sarge, K.D. & Abravaya, K. (1992) Transcriptional regulation of heat shock genes. *J. Biol. Chem.* **267**, 21987–21990.
7. Jones, P.G., VanBogelen, R.A. & Neidhardt, F.C. (1987) Induction of proteins in response to low temperature in *Escherichia coli*. *J. Bacteriol.* **169**, 2092–2095.
8. Jones, P.G. & Inouye, M. (1994) The cold-shock response — a hot topic. *Mol. Microbiol.* **11**, 811–819.
9. Broeze, R.J., Solomon, C.J. & Pope, D.H. (1978) Effect of low temperature on *in vivo* and *in vitro* protein synthesis in *Escherichia coli* and *Pseudomonas fluorescens*. *J. Bacteriol.* **134**, 861–874.
10. Friedman, H., Lu, P. & Rich, A. (1969) Ribosomal subunits produced by cold sensitive initiation of protein synthesis. *Nature (London)* **233**, 909–913.
11. Friedman, H., Lu, P. & Rich, A. (1971) Temperature control of initiation of protein synthesis in *Escherichia coli*. *J. Mol. Biol.* **61**, 105–121.
12. Jones, P.G., Cashel, M., Glaser, G. & Neidhardt, F.C. (1992) Function of the relaxed-like state following temperature downshifts in *Escherichia coli*. *J. Bacteriol.* **174**, 3903–3914.
13. Araki, T. (1991) Changes in rates of synthesis of individual proteins in a psychrophilic bacterium after a shift in temperature. *Can. J. Microbiol.* **37**, 840–847.
14. Av-Gay, Y., Aharanowitz, Y. & Cohen, G. (1992) Streptomyces contain a 7.0 kDa cold-shock protein. *Nucleic Acids Res.* **20**, 5478.
15. Cloutier, J., Prévost, D., Nadeau, P. & Antoun, H. (1992) Heat and cold shock protein synthesis in arctic and temperate strains of Rhizobia. *Appl. Envir. Microbiol.* **58**, 2846–2853.
16. Goldstein, J., Politt, N.S. & Inouye, M. (1990) Major cold shock protein of *E. coli*. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 283–287.
17. Lee, S.J., Xie, A., Jiang, W., Etchegray, J.-P., Jones, P.G. & Inouye, M. (1994) Family of the major cold-shock protein, CspA (CS7.4) of *Escherichia coli*, whose members show a high sequence similarity with the eukaryotic Y-box binding proteins. *Mol. Microbiol.* **11**, 833–839.
18. Maniak, M. & Nellen, W. (1988) A developmentally regulated membrane protein gene in *Dictyostelium discoideum* is also induced by heat shock and cold shock. *Mol. Cell. Biol.* **8**, 153–159.
19. Potier, P., Drevet, P., Gounot, A.M. & Hipkiss, A.R. (1990) Temperature- dependent changes in proteolytic activities and protein composition in psychrotrophic bacterium *Arthrobacter globiformis* S155. *J. Gen. Microbiol.* **136**, 283–291.
20. Roberts, M.E. & Innis, W.E. (1992) The synthesis of cold shock proteins and cold acclimation proteins in the psychrotrophic bacterium *Aquaspirillum arcticum*. *Curr. Microbiol.* 25, 275–278.
21. Willimsky, G., Bang, H., Fischer, G. & Marahiel, M.A. (1992) Characterization of *cspB*, a *Bacillus subtilis* inducible cold shock gene affecting cell viability at low temperatures. *J. Bacteriol.* **174**, 6326–6335.
22. Jones, P.G., Krah, R., Tafuri, S.R. & Wolfe, A.P. (1992) DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. *J. Bacteriol.* **174**, 5798–5902.
23. La Teana, A., Brandi, A., Falconi, M., Spurio, R., Pon, C.L. & Guarlezi, C.O. (1991) Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10907–10911.
24. Qoronfleh, M.H., Debouck, C. & Keller, J. (1992) Identification and characterization of novel low-temperature-inducible promoters of *Escherichia coli*. *J. Bacteriol.* **174**, 7902–7909.
25. Jiang, W., Jones, P. & Inouye, M. (1993) Chloramphenicol induces the transcription of the major cold shock gene of *Escherichia coli*, *cspA*. *J. Bacteriol.* **175**, 5824–5828.
26. VanBogelen, R.A. & Neidhardt, F.C. (1990) Ribosomes as sensors of heat and cold shock in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5589–5593.
27. Das, A. (1992) How the phage lambda N gene product suppresses transcription termination: communication of RNA polymerase with regulatory proteins mediates by signals in nascent RNA. *J. Bacteriol.* **174**, 6711–6716.
28. Friedman, D.I. (1984) Interactions of bacteriophage and host macromolecules in the growth of bacteriophage λ. *Microbiol. Rev.* **48**, 299–325.
29. Grunberg-Manago, M. (1987) Regulation of the expression of aminoacyl-tRNA synthetases and translation factors; in *Escherichia coli and Salmonella typhimurium: cellular and molecular biology* (Neidhardt, F.C., ed.) pp. 1386–1409, Amer. Soc. Microbiol., Washington, D.C.
30. Guarlezi, C.O. & Pon, C.L. (1990) Initiation of mRNA translation in prokaryotes. *Biochemistry* **29**, 5881–5889.
31. Nakamura, Y. & Mizusawa, S. (1985) *In vivo* evidence that the *nusA* and *infB* genes of *E. coli* are part of the same multi-gene operon which encodes at least four proteins. *EMBO J.* **4**, 527–532.
32. Takata, R.T., Mukai, T. & Hori, K. (1985) Attenuation and processing of RNA from the

rpsO-pnp transcription unit of *Escherichia coli*. *Nucleic Acids Res.* **13**, 7289–7286.

33. Donovan, W.P. & Kushner, S.R. (1986) Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 120–124.
34. Walker, G.C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol. Rev.* **48**, 60–93.
35. Schmid, M.B. (1990) More than just "histone-like" proteins. *Cell* **63**, 451–453.
36. Sugino, A., Peebles, C.L., Kreuzer, K.N. & Cozarelli, N.R. (1977) Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4767–4771.
37. Tanabe, H., Goldstein, J., Yang, M. & Inouye, M. (1992) Identification of the promoter region of the *Escherichia coli* major cold shock gene, *cspA*. *J. Bacteriol.* **174**, 3867–3873.
38. Wistow, G. (1990) Cold shock and DNA binding. *Nature (London)* **344**, 823–824.
39. Chatterjee, S., Jiang, W., Emerson, D. & Inouye, M. (1993) The backbone structure of the major cold-shock protein CS7.4 of *Escherichia coli* in solution includes extensive β-sheet structure. *J. Biochem.* **114**, 663–669.
40. Schindelin, H., Jiang, W., Inouye, M. & Heinemann, U. (1994) Crystal structure of CspA, the major cold shock protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5119–5123.
41. Newkirk, K., Feng, W., Jiang, W., Tejero, K., Emerson, S.D., Inouye, M. & Montelione, G.T. (1994) Solution NMR structure of the major cold shock protein (CspA) from *Escherichia coli*: Identification of the binding epitope for DNA. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5114–5118.
42. Landsman, D. (1992) RNP-1, and RNA-binding motif is conserved in DNA-binding cold shock domain. *Nucleic Acids Res.* **20**, 2861–2864.
43. Schnuchel, A., Witschech, R., Czisch, M., Herrier, M., Willimsky, G., Graumann, P., Marahiel, M.A. & Holah, T.A. (1993) Structure in solution of the major cold-shock protein from *Bacillus subtilis*. *Nature (London)* **364**, 169–171.
44. Schindelin, H., Marahiel, M.A. & Heinemann, U. (1993) Universal nucleic acid binding domain revealed by crystal structure of the *B. subtilis* major cold-shock protein. *Nature (London)* **364**, 164–168.
45. Araki, T. (1991) The effect of temperature shifts on protein synthesis by the psychrophilic bacterium *Vibrio* sp. strain ANT-300. *J. Gen. Microbiol.* **137**, 817–826.
46. Taura, T., Kusukawa, N., Yura, T. & Ito, K. (1989) Transient shut off of *Escherichia coli* heat shock protein synthesis upon temperature shift down. *Biochem. Biophys. Res. Commun.* **163**, 438–443.
47. Cashel, M. & Rudd, K.E. (1987) The stringent response; in *Escherichia coli and Salmonella typhimurium: cellular and molecular biology* (Neidhardt, F.C., ed.) pp. 1410–1438, Amer. Soc. Microbiol. Washington, D.C.
48. Pao, C.C. & Dyess, B.T. (1981) Stringent control of RNA synthesis in the absence of guanosine 5'-diphosphate-3'-diphosphate. *J. Biol. Chem.* **256**, 2252–2257.
49. Mackow, E.R. & Chang, F.N. (1983) Correlation between RNA synthesis and ppGpp content in *Escherichia coli* during temperature shifts. *Mol. Gen. Genet.* **192**, 5-9.
50. Julseth, C.R. & Inniss, W.E. (1990) Induction of protein synthesis in response to cold shock in the psychrotrophic yeast *Trichosporon pullulans*. *Can. J. Microbiol.* **36**, 519–524.
51. Kondo, K. & Inouye, M. (1991) TIP1, a cold shock-inducible gene of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **266**, 17537–17544.
52. Kondo, K. & Inouye, M. (1992) Yeast NSR1 protein that has structural similarity to mammalian nucleolin is involved in pre-rRNA processing. *J. Biol. Chem.* **267**, 16252–16258.
53. Kondo, K., Kowalski, L. & Inouye, M. (1992) Cold shock induction of yeast NSR1 protein and its role in pre-rRNA processing. *J. Biol. Chem.* **267**, 16259–16265.
54. Westphal, M., Müller-Taubenberger, A., Noegel, A. & Gerisch, G. (1986) Transcript regulation and carboxyterminal extension of ubiquitin in *Dictyostelium discoideum*. *FEBS Lett.* **209**, 92–96.
55. Salerno, G.L. & Pontis, H.G. (1988) Raffinose synthesis in *Chlorella vulgaris* cultures after a cold shock. *Plant Physiol.* **89**, 648–651.