J. Biosci., Vol. 21, Number 2, April 1996, pp 149–160. © Printed in India.

Stress response in pathogenic bacteria

RUKHSANA CHOWDHURY, GAUTAM K SAHU and JYOTIRMOY DAS*

Biophysics Division, Indian Institute of Chemical Biology, 4, Raja S C Mullick Road, Jadavpur, Calcutta 700 032, India

MS received 23 September 1995; revised 16 December 1996

Abstract. Bacterial pathogens survive under two entirely different conditions, namely, their natural environment and in their hosts. Response of these pathogens to stresses encountered during transition from the natural environment to human hosts has been described. The virulence determinants of pathogenic bacteria are under the control of transcriptional activators which respond to fluctuations in growth temperature, osmolarity, metal ion concentration and oxygen tension of the environment. The regulation of stress induced genes may occur at the level of transcription or translation or by post-translational modifications. Under certain stress conditions local changes in the superhelicity of DNA induce or repress genes. In addition to their role in survival of bacteria under stressful situations, the stress induced proteins are also implicated in the manifestation of pathogenicity of bacterial pathogens *in vivo*.

Keywords. Temperature stress; virulence genes; heat shock proteins; anaerobiosis; osmotic stress; metal ions.

1. Introduction

Pathogenic bacteria, unlike innocuous commensals alternate between free living and host associated states. The physico-chemical parameters encountered by the bacteria in these two states are very different and exert different demands and stresses on the bacterial cell. Bacterial pathogens have evolved highly sophisticated mechanisms for sensing external conditions and respond by altering the pattern of gene expression with activation of a set of genes whose products assist in survival and turning off those the products of which are not necessary in a particular environment. These sensor-activator systems allow the bacteria to monitor environmental parameters which distinguish host from external environment and adjust gene expression accordingly, particularly by induction of virulence factors (Albright *et al* 1989; Parkinson and Kofoid 1992). The expression of virulence genes is controlled by regulatory systems in such a manner that the virulence factors are expressed at different stages of the infection process dictated by the changing micro-environment of the host as a consequence of the pathophysiology of infection. Accordingly, mutations in some of the regulatory systems attenuate virulence of several bacterial species (Dorman *et al* 1989).

The environmental control of regulatory mechanisms is mediated by complex processes both at the level of transcription and translation. Moreover, stress conditions like changes in the osmolarity of the growth medium, anaerobiosis and temperature which pathogenic bacteria encounter upon entry into the host, can control gene expression by inducing changes in DNA topology which can provide an overlap between response to different environmental stimuli (Dorman 1991).

*Corresponding author (Fax, 091-33 473 0350/0284; Email, iicb%sirnetc@sirnetd.ernet.in).

This review describes how bacterial pathogens infecting humans respond to stress situations encountered during transition from natural environment to the host with special reference to induction of virulence determinants which is of particular interest in the study of microbial pathogenesis.

2. Temperature stress

2.1 *Induction of virulence genes*

The first signal to an invading bacteria on entry into the host is an increase in temperature from that of the environment to the physiological temperature of the human body (37°C). In *salmonellae, shigellae, yersinae, Bordetella pertusis* (Maurelli 1989), *Borrelia burgdorferi* (Cluss and Boothby 1990), *Listeria monocytogens* (Wachter *et al* 1992) and several other pathogenic organisms, the virulence gene cassettes are switched on at 37°C. In many of these pathogens, the virulence determinants are under the control of transcriptional activators which respond to fluctuations of growth temperature leading to an enhanced expression of virulence genes at 37°C. In *L. monocytogenes,* a Gram-positive facultative intracellular pathogen, the activation of virulence genes is under the control of the transcriptional activator PrfA (Wachter *et al* 1992). The gene encoding PrfA is transcribed from its own promoter as a monocistronic transcript at 30°C. At 37°C, in addition to the monocistronic transcript, the *prfA* gene is also transcribed from a different promoter as a bicistronic message comprising of the *prf* and the *pic* (phosphatidylinositol-specific phospholipase C) genes. Hence the *prfA* gene is transcribed from two different promoter regions at higher growth temperatures and it is the bicistronic transcript that is thermally regulated. At 30°C none of the virulence genes under the control of *prfA* are transcribed although at 37°C all of them are expressed. The repression of transcription of the PrfA regulated virulence genes at low temperatures might be due to either limitation of other yet unknown cellular components which may act in concert with PrfA or temperature dependent conformational change in the PrfA protein.

A mechanism of temperature regulation of virulence genes similar to that in *L. monocytogenes* may be operative in *Yersinia pestis* and *Shigella flexineri* (Barve and Straley 1990; Tobe *et al* 1991; Hoe *et al* 1992). *Icr*F gene product of *Y. pestis* and the *vir*F gene product of *S. flexineri,* both belonging to the *araC* family, are the transcriptional activators of virulence genes of these organisms. Expression of the virulence genes under the control of LcrF and VirF are coordinately regulated by growth temperature. Although the levels of transcription of the *Icr*F and *vir*F genes are not significantly affected by changes in temperature, the induction of virulence genes is more at 37°C but not at lower temperature.

Alternative mechanisms for activation of the transcription factors of virulence genes have been described in *Y. enterolytica* and *V. cholerae.* In *Y. enterolytica,* the *vir*F gene is the transcriptional activator of virulence genes. The transcription of this gene itself is regulated by temperature (Cornelis *et al* 1989). There are evidences which suggest that the increase in relative abundance of *vir*F message with increasing temperature may be due to alternations of DNA superhelicity (Dorman 1991). In contrast to other pathogenic organisms the virulence genes in *V. cholerae, Yersinia pseudotuberculosis, Yersinia enterocolitica* are optimally expressed at 30°C and reduced at 37°C (Isberg *et al* 1988; Parsot and Mekalanos 1990; Pierson and Falkow 1990). It has been

proposed that the divergent transcription of a *htp*G-like heat shock gene in *V. cholerae* leads to a proportionate decrease in the expression of *tox*R, coding for a transmembrane DNA-binding protein that positively regulates transcription of the genes for cholera toxin and other virulence determinants (Parsot and Mekalanos 1990). ToxR represses several gene functions which presumably include some necessary for chemotaxis and motility (Miller and Mekalanos 1988; Strauss 1995). Since motility is involved in establishing the infection, it is possible that at an early stage of the infection process, ToxR remains repressed and once the bacterium reaches the site of colonization, as yet unidentified environmental signal(s) at the surface of the mucosal epithelium may activate ToxR leading to the expression of virulence genes.

2.2 *Induction of heat shock genes*

In addition to regulation of virulence genes in pathogenic organisms, temperature stress also induces the ubiquitous heat shock response involving the expression of a set of heat shock proteins (HSPs). Induction of HSPs occurs primarily at the level of transcription and in *E. coli,* the product of the *rpoH* (*htp*R) gene, a σ-factor (σ^{32}), is required for the transcription of heat shock genes (Yura *et al* 1993). σ^{32} binds to the core RNA polymerase and recognizes the heat shock promoters which differ signify-cantly from regular promoters with respect to – 35 (consensus TCTCnCCCTTGAA) and –10 (consensus CCCCATnTA) regions and the length of the spacer (13–17 nucleotides) between these regions. The heat inducibility of the heat shock genes is due to availability of a greater number of σ^{32} to associate with core RNA polymerase at higher temperatures. At low temperature, σ^{32}, present at a basal level in the cell, does maintain a low level of transcription of most of the heat shock genes. The concentration of σ^{32} increases by about 20-fold within the first five min after upshift of temperature from 30° to 42°C and then decreases to a steady state level characteristic of the elevated temperature. This increase is primarily due to enhanced rate of translation of the *rpo*H mRNA and increased stability of the protein at high temperature. The increase in the rate of translation has been attributed to an alteration in the secondary structure of *rpo*H mRNA which favours efficient ribosome binding and initiation of translation. σ^{32} interacts with DnaK-DnaJ-GrpE chaperones and the complex is susceptible to cleavage by the protease FtsH (Tomoyasu *et al* 1995). This makes σ^{32} highly unstable (half life of 1 min at 30°C) at low temperatures. At high temperatures, the DnaK chaperone machinery is sequestered by binding to unfolded polypeptides that accumulate within the cell following temperature upshift leaving free σ^{32}. FtsH protease cleaves free σ^{32} *in vitro* in the presence of ATP (Tomoyasu *et al* 1995). It is possible that at high temperature, the free σ^{32} has greater affinity towards core RNA polymerase and the cleavage by FtsH is hindered, thereby increasing the half life of σ^{32} to about 4–5 min at 42°C (Bukau 1993). During the adaptation period, the level of DnaK increases with the concomitant increase in the amount of σ^{32} complexed with DnaK which are eventually cleaved by FtsH reducing the half life of σ^{32}. Under extreme heat stress conditions, another σ factor σ^{E} (σ^{24}), a 24 kDa protein is induced which controls the expression of a set of genes including *htr*A, *htr*C and *rpo*H (Erickson and Gross 1989; Raina *et al* 1985; Rouviere *et al* 1995).

Temperature regulation of expression of virulence genes is distinct from the heat shock response in the character of the induction process. In heat shock response, the initial large increase in transcription of the heat shock genes is transient and is followed

by the adaptation phase when the level of induction falls to a lower steady state value characteristic of the new elevated temperature (Yura *et al* 1993). Induction of the virulence genes is more directly coupled to temperature and does not decrease unless temperature is lowered. Thus, the mechanism of negative regulation of the heat shock response postulated to account for the decrease in expression of the heat shock genes after the initial increase (Bukau 1993) is not applicable to temperature dependent virulence gene expression, although there appears to be a basic Similarity, in principle, in the mechanism of positive regulation of these two processes, i.e., activation of a transcriptional factor by increased temperature which recognizes certain features present in the promoters of the genes it controls. Moreover, the thermometer, i.e., the basic temperature sensing system in the cell has not been identified in either case although there is an evidence which suggests that DnaK may be the cellular thermometer (Craig and Gross 1991).

Major HSPs from bacteria to humans are a highly conserved class of proteins and represent a significant proportion of the total protein content of all living cells. Many of the HSPs are constitutively present in the cell even under no-stress situation and perform important house-keeping functions (Craig 1993). The major HSPs (DnaK, DnaJ, GrpE, GroEL) are molecular chaperones that assist in correct folding and assembly of proteins and are involved in diverse cellular processes including DNA replication, RNA transcription, flagella synthesis and UV mutagenesis (Yura *et al* 1993). GroEL together with GroES facilitates protein translocation across membrane barriers and possibly also secretion. The fundamental functions of these HSPs are to prevent protein denaturation and to reactivate partially denatured proteins. Non-repairable denatured proteins are degraded by another class of HSFs which represent either an ATP dependent protease (Lon, La) or a catalytic (ClpP, Ti) or regulatory (ATPase) subunit (ClpB) of another protease Clp (Craig 1993).

In *Pseudomonas aeruginosa,* the major σ-factor, σ^{87} was shown to be a HSP and is immunologically related to σ^{70} of *E. coli* (Allan *et al* 1988). A 40 kDa protein has been reported to be associated with RNA polymerase purified from heat shocked cells. This protein may represent the heat shock σ-factor of *P. aeruginosa.* Under mild heat shock conditions (37°–42°C) DnaK, GroEL and GroES analogous of *M. tuberculosis* are induced (Young and Grabe 1991). When the temperature was raised to 48°C, the amount of the 65 kDa GroEL was drastically reduced and several HSPs of molecular masses ranging from 90–15 kDa Were induced. The pattern of HSP synthesis is dependent on the severity of the heat stress with two distinct phases being discernible in the heat shock response. Unlike in *L. monocytogenes,* where expression of heat shock genes in clinical isolates has some correlation with virulence properties (Sokolovic *et al* 1990), no significant difference was observed when stress induced proteins were examined in *V. cholerae* (Sahu *et al* 1994) and *M. tuberculosis* (Young and Garbe 1991) strains with varying degree of virulence. Sixteen HSPs have been identified in *V. cholerae* (Sahu *et* al 1994). One of the major low molecular mass HSP, a 16 kDa protein is preferentially degraded following shift down of temperature. This protein is induced at a much lower level at high temperature in cells maintained in the laboratory for a prolonged period. These laboratory maintained cells showed increased sensitivity to heat and low pH. During laboratory subculturing of *V. cholerae* cells, several metabolic functions are altered along with a reduction in toxinogenicity of the cells. All these functions can be restored by a single passage of the cells in guinea pig (Roy *et al* 1982). The synthesis of the 16 kDa protein resumed following a single animal passage along

with reduction of the heat sensitivity of the cells (Sahu *et al* 1994). Whether the 16 kDa protein has any role in virulence is yet to be investigated. This protein is antigenic and antibodies against this inner membrane protein could be detected in sera of convalescent cholera patients. The only HSP located at the outer membrane of *V. cholerae* is a 23 kDa protein. The gene coding for a σ-factor, analogous to the *rpo*H gene product of *E. coli,* has been cloned and its nucleotide sequence determined (G K Sahu, R Chowdhury and J Das, unpublished observation). The deduced amino acid sequence of the *rpo*H like gene of *V. cholerae* has more than 80% homology with its *E. coli* counterpart (Landick *et al* 1984). The *rpo*H genes encoding σ^{32} homologs have also been sequenced from *Citrobacter freundii, Pseudomonas aeruginosa* and five other Gram-negative bacteria (Garvin and Hardris 1989; Nakahigashi *et al* 1995).

HSPs participate in immune response to bacterial infections and development of autoimmune diseases (Murray and Young 1992). Different classes of HSPs from different bacteria can directly induce cytokine expressions and secretion in macrophage (Retzlaff *et al* 1994). Hsp60 has been found to be a common antigen of many bacterial pathogens including species of *Pseudomonas, Mycobacterium, Borrelia, Salmonella, Legionella, Coxiella* and *Rickettsia* (Shinnick 1991). In *Borellia burgdorferi,* there are two HSP60 of molecular masses 60 and 66 kDa which have been implicated in developing autoimmune pathologies such as arthritis (Carreiro *et al* 1990). The HSP70 of the organism, however, did not react with immune sera from Lyme disease patients. HSP60 class of proteins serve as immunodominant targets of α, β and γ, δ classes of T-lymphocytes and have been used to provoke immunological protection against *Mycobacterium* and *Legionella* (Blander and Horowitz 1993; Silva and Lowrie 1994). It has been shown that *S. typhimurium* which had been phagocytized by murine macrophages, markedly increase their HSP level, including HSP60 and HSP70 (Buchmeir and Heffron 1990). This abundance of HSP may be one reason for their immunodominance. However, using a different macrophage Abshire and Neidhardt (1993) failed to detect induction of these HSPs following phagocytosis by *S. typhimurium.* It has been postulated that since HSP60 is highly conserved, the host may frequently encounter this antigen through infection with various other microorganisms thereby constantly boosting the immune response to HSP (Kaufmann *et al* 1991). Surprisingly the HSP70 and not HSP60 of *V. cholerae* reacted with sera from convalescent cholera patients. The major antigen in *V. cholerae* is a 23 kDa HSP located in the outer membrane (Sahu *et al* 1994).

3. Oxygen stress

The expression of adherence and invasion factors of several pathogenic bacteria is regulated by oxygen concentration. High oxygen usually represses whereas low oxygen induces invasiveness. During switch from aerobic to anaerobic growth condition, a set of genes are induced and some genes are repressed (Iuchi and Lin 1991). In *E. coli* two regulatory mechanisms have been identified which control the expression of these genes. One regulatory network is the Fnr (fumerate-nitrate reductase)-dependent control in response to anaerobiosis. Fnr activates the transcription of several respiratory genes such as fumarate reductase (*frd*) (Jones and Gunsalas 1987; Lambden and Guest 1976), dimethyl sulphoxide-triethylamine-N-oxide reductase (*dms*) (Cotter and Gunsalas 1989) and nitrate reductase (*nar*) (Stewart 1982; Chippaux *et al* 1982) and

represses the expression of cytochrome d (*cyd*) operon (Cotter and Gunsalas l992 Cotter *et al* 1990). The *frd* and *nar* gene products are required for the reduction of fumarate and nitrate so that they can serve as alternative electron acceptors for oxidative phosphorylation even in the absence of oxygen. Under semi-anaerobic conditions, the cytochrome d oxidase, which has low K_m value for oxygen is induced under the control of ArcA–ArcB, a two component sensor regulator system responsive to a shift from aerobic to anaerobic growth. ArcA–ArcB-dependent regulation represses transcription of several genes involved in aerobic metabolism.

The response to switch from aerobic to anaerobic growth conditions has been examined in detail in *Salmonella* sp and a number of invasion genes that are expressed under low oxygen environment have been identified. In *S. typhimurium* a gene, *org*A, has been identified, mutation in which disrupts the ability of the organism to enter tissue culture cell (Jones and Falkow 1994). The gene does not have significant similarity to other sequences present in the Gene Bank. The upstream of the *org*A ORF has two direct repeats similar to those identified for the *fnr*-dependent promoters. These repeats overlap with the putative promoter of *org*A.

4. Osmotic stress

For a pathogenic bacterium which passes from environmental waters to the human body for infection, osmolarity is an important criterion to distinguish between the external and host associated environments. Osmolarity of an aqueous environment is thought to be no greater than that equivalent to 0·06 M NaCl while in the intestinal lumen the osmolarity is much higher (equivalent to 0·3 M NaCl) and in the blood stream the bacteria encounters an osmolarity equivalent to about 0·15 M NaCl. Thus, an increase in osmolarity is associated with expression of virulence factors in many pathogenic organisms. In *S. flexineri,* expression of the plasmid located *vir* genes which are necessary for invasion of epithelial cells is markedly enhanced under conditions of high osmoIarity (0·15 M NaCl). The two component regulatory system OmpR-EnvZ encoded by the OmpB locus is responsibIe for sensing and responding to the signal and controls the expression of the *vir* genes as well as the chromosomal *omp*F-*omp*C genes enconding the osmoregulated porins which are probably necessary for intracellular survival (Bernardini *et al* 1990). Thus, *omp*B deletion mutants of *S. flexineri* are defective with respect to both invasion and survival within the host tissues. However, *envZ* mutations alone caused a decrease in expression of *vir* genes, but did not abolish their derepression under high osmolarity. These results suggested that although EnvZ is a major factor required for optimal expression of virulence genes, it is not the only component involved in the response of *vir* genes to osmolarity and cross talk between OmpR and an alternative component could restore osmoinduction of *vir* genes in EnvZ deficient mutants. Recent reports suggest that OmpC may actually be involved in the invasion of epithelial cells by *S. flexineri* since a mutation in the *omp*C gene impaired colonization of epithelial cells (Bernardini *et al* 1993). Interestingly, OmpC is expressed constitutively under conditions of high and low osmolarity in *S. flexineri,* whereas in *E. coli* and *S. typhimurium* its expression is increased at high osmolarity. In *S. typhimurium* osmolarity affects the expression of the OmpC and OmpF porins and both the *omp*C and *omp*F genes have been shown to contain supercoiling sensitive promoters (Graeme-Cook *et al* 1989). Unlike in *S. flexineri,* the invasion genes (*inv*ABC

operon) of *S. typhimurium* are independent of *omp*R which controls the osmoinducibility of *omp*C and *omp*F, although they too exhibit osmolarity dependent expression and transcription of *inv*A (the proximal gene of *inv*ABC operon) was about 8-fold higher when cells were grown in medium of high osmolarity (Galan and Curtiss 1990). Since antibiotics that reduce the superhelicity of DNA caused a decrease in the expression of *inv*A, osmolarity may control expression of invasion genes by changing DNA supercoiling. This is further supported by the observation that *inv*A expression was reduced at low temperatures, a condition known to cause a reduction in the linking number of DNA. Although expression of the invasion genes is independent of *omp*R, mutations in *omp*R lead to an attenuation of virulence that cannot be accounted for only by changes in the concentrations of OmpC and OmpF, suggesting that other genes under the control of the *omp*R regulatory system may contribute to *Salmonella* virulence (Chatfield *et al* 1991). It has been shown that in *S. typhimurium*, the *omp*R gene is involved in the osmolarity dependent regulation of synthesis of the Vi capsular polysaccharide (Pickard *et al* 1994). Synthesis of Vi is reduced at osmolarity equivalent to 300–400 mM NaCl indicating that down regulation of Vi probably occurs in the gut facilitating interaction with epithelial cells whereas its up regulation at osmolarity equivalent to 150 mM NaCl similar to that present in the blood stream allows survival of the organism at this stage of infection as it is known that Vi is important for survival of *S. typhimurium* in blood (Looney and Steigbigel 1986).

P. aeruginosa, which infects cystic fibrosis patients, synthesizes alginate, a capsular polysaccharide, necessary for maintenance of virulence of the organism. High osmolarity in the lungs of cystic fibrosis patients is one of the signals that contribute to the increased transcription of *alg*D gene encoding GDP mannose dehydrogenase, a primary controlling factor in the alginate biosynthesis. Activation of *alg*D under high osmolarity condition is dependent on the product of the *alg*R gene which has high sequence homology to *E. coli omp*R. In fact *E. coli* OmpR can activate *alg*D promoters to a level comparable to the activation by AlgR under similar conditions of high osmolarity (Deretic *et al* 1989). The *alg* operon is also dependent on a histone like protein (AlgP) for its optimal expression. Together with the observation that increase in growth medium osmolarity elevated supercoiling of reporter plasmids, this data suggests that DNA topology regulated by histone like proteins may be an important regulatory factor in the transcriptional control of the *alg* genes (Deretic *et al* 1991).

In *V. cholerae,* expression of virulence factors is also dependent on osmolarity of the growth medium and is maximum at osmolarity equivalent to about 60 mM NaCl. This value is much lower than the osmolarity of the intestinal lumen (equivalent to about 0·3 M NaCl), the site of infection by *V. cholerae.* The C-terminal periplasmic domain of ToxR, the transcriptional activator of virulence genes in *V. cholerae,* is probably the osmosensor since replacement of this part of the protein (by fusion with PhoA) rendered the protein constitutively active even under high salt conditions (Miller *et al* 1987). The cytoplasmic N-terminal domain of the protein has homology with OmpR (Miller *et al* 1987). In addition to cholera toxin and pilus production, ToxR also controls the relative proportions of the two major outer membrane proteins of *V. cholerae,* OmpT and OmpU (Miller and Mekalanos 1988). Conditions that activate ToxR result in increase in OmpU expression with concomitant activation of cholera toxin and pilus production. Expression of OmpT always follows a pattern opposite to that of OmpU and other virulence factors and appears to be negatively regulated by ToxR. This complex regulation of activation of certain genes and repression of others

by ToxR probably reflects a fine tuning of gene expression during infection to respond to different demands at different stages of the infection process.

5. Metal ion stress

Free iron is extremely limited in the tissues and fluids of mammalian systems (Bullen 1973). Iron is an essential element for bacterial growth and many pathogenic bacteria have evolved highly efficient iron scavenging systems which are regulated in response to the iron status of the environment. In addition, low iron concentration leads to the increased synthesis of virulence determinants in several pathogenic bacteria including shiga-like toxin of enteropathogenic *E. coli* (Calderwood and Mekalanos 1987), shiga toxin of *S. dysenterie,* diptheria toxin of *Cornybacterium diptheriae* (Boyd *et al* 1990), exotoxin A of *P. aeruginosa* (Bjorn *et al* 1978) and so on. The molecular mechanism of iron regulation of gene expression has been thoroughly studied in *E. coli.* The coordinate expression of iron regulated genes involves the Fur protein as repressor and iron as co-repressor. The repressor-co-repressor complex binds to operator sites within the promoters of the iron regulated genes (Schaffer *et al* 1985). A consensus DNA binding site for the Fur protein consists of a 21 bp dyad symmetric sequence (Calderwood and Mekalanos 1988). This sequence, present upstream of the gene encoding the shiga-like toxin (*slt*) in *E. coli is* responsible for the Fur dependent repression of shiga-like toxin at high iron concentration (Calderwood and Mekalanos 1987). A large number of pathogenic bacteria including *S. typhimurium, Serratia mercescens, Y. pestis*, *V. cholerae* have Fur like iron regulatory systems (Ernst *et al* 1978; Staggs and Perry 1991; Poole and Braun 1988; Litwin *et al* 1992). The transcription of *irg*A gene, coding for a major iron regulated outer membrane protein of *V. cholerae,* is repressed at high iron concentration by the Fur protein of *V. cholerae.* The promoter of *irg*A gene contains a 19 bp dyad symmetry that is similar to the Fur binding sites of *E. coli* (Goldberg *et* al 1990). Unlike iron regulated genes of *E. coli,* transcription of *V. cholerae irg*A also requires the positive transcriptional activator protein IrgB (Goldberg *et al* 1990a, b). *irg*B is divergently transcribed from *irg*A and the promoters of *irg*A and *irg*B overlap the same Fur box. The transcription of *irgB* is itself negatively regulated by iron, It was shown by Tn-*pho*A insertion mutation of *irg*A that the product of this gene is necessary for virulence of *V. cholerae* in an animal model. Competition of the mutant and wild type strains *in vivo* leads to a competitive index of 0·11 suggesting that the IrgA protein may be necessary for colonization of the mice intestine by *V cholerae.* However, high level induction of IrgA could not be detected in rabbit and mice intestine following infection of *V. cholerae* (Camelli *et al* 1994). When grown in low iron medium, five new outer membrane proteins could be detected of which one might serve as vibriobactin, a catechol type siderophore receptor for iron transport (Stoebner and Payne 1988). Synthesis of both haemolysin and vibriobactin which are coordinately regulated are repressed by the addition of iron in the medium. The ability of *V. cholerae* to acquire iron from the host is linked to virulence and disruption of either heme utilization or vibriobactin uptake system reduces the ability of the organism to colonize the intestine (Henderson and Payne 1994).

Calcium plays a role in the regulation of virulence genes in *Y. pestis* (Barve and Straley 1990). While the growth of this organism at 37°C requires mM levels of calcium, the virulence genes are expressed only in the absence of calcium under *in vitro*

conditions. How these cells divide and express virulence determinants under *in vivo* condition is not clear.

6. Conclusion

Depending upon the stress condition, a set of bacterial genes is induced and several functions are repressed. In several organisms, some repressed proteins are also required for virulence. For example, in *S. typhimurium,* the *pho*P repressed *prg*H locus, which is composed of four genes, contributes to pathogenesis (Miller and Mekalanos 1990). In *B. pertusis* the expression of a number of outer membrane proteins is down-regulated in the virulence-activated state (Beattie *et al* 1992). In the present report only the induced genes functions and their role in virulence determination have been discussed.

Bacterial responses to different stress conditions have been studied mostly under *in vitro* conditions. Although the results obtained from these studies have provided useful information on survival under stress conditions and expression of virulence genes, they might not truly reflect the *in vivo* situation where host factors also contribute to establishment of the organism during infection. For a better understanding of the host-parasite interaction it is desirable to delineate the bacterial functions that are specifically expressed under *in vivo* conditions and to assign their role in pathogenesis. Several approaches are now being adopted to identify *in vivo* expressed genes and examine their functions. The results from such analysis will furnish a clearer picture of how human pathogens adapt themselves to *in vivo* stress situations and provide a better insight into the molecular basis of pathogenicity.

Acknowledgements

This work was supported by the Department of Biotechnology [Grant No. BT/TF/15/03/91], New Delhi, to JD and Council of Scientific and Industrial Research Young Scientist Grant to RC. GKS is grateful to the Council of Scientific and Industrial Research, New Delhi for a predoctoral fellowship.

References

Abshire K Z and Neidhardt F C 1993 Analysis of proteins synthesized by *Salmonella typhimurium* during growth within a host macrophage; *J. Bacteriol.* **175** 3734–3743

Albright L M, Huala E and Ausubel F M 1989 Prokaryotic signal transduction mediated by sensor and regulator protein pairs; *Annu. Rev. Genet.* **23** 311–336

Allan B, Linseman M, MacDonald L A, Lam J S and Kropinski A M 1988 Heat shock response of *Pseudomonas aeruginosa; J. Bacteriol.* **170** 3668–3674

Barve S S and Straley S C 1990 *IcrR,* a low Ca^{2+} response locus with Ca^{2+} dependent functions in *Yersinia pestis; J. Bacteriol.* **172** 4661–4671

Beattie D T, Shahin R and Mekalanos J J 1992 A *vir*-repressed gene of *Bordetella pertussis* is required for virulence; *Infect. Immun* **60** 571–577

Bernardini M L, Fontaine A and Sansonetti P J 1990 The two component regulatory system OmpR-EnvZ controls the virulence of *Shigella flexineri; J. Bacteriol.* **172** 6274–6281

Bernardini M L, Sanna M G, Fontaine F and Sansonetti P J 1993 OmpC is involved in invasion of epithelial cells by *Shigella flexineri; Infect Immun*. **61** 3625–3635

Bjorn M J, Iglewski B H, Ives S K, Sadoff J C and Vasil M L 1978 Effect of iron on yields of exotoxin A in cultures of *Pseudomonas aeruginosa* PA-103; *Infect. Immun.* **19** 785–791

Blander S J and Horwitz M A 1993 Major cytoplasmic membrane protein of *Legionella pneumophila,a* genus common antigen and member of the *hsp*60 family of heat shock proteins induces protective immunity in a guinea pig model of Legionnaires disease; *J. Clin. Invest.* **91** 717–723

Boyd J, Oso M N and Murphy J R 1990 Molecular cloning and DNA sequence analysis of a diphtheria tox iron-dependent regulatory element (*dtxR*) from *Corynebacterium diphtheriae; Proc. Natl. Acad. Sci. USA* **87** 5968–5972

Buchmeir N A and Heffron F 1990 Induction of *Salmonella* stress proteins upon infection of macrophages, *Science* **248** 730–732

Bukau B 1993 Regulation of the *Escherichia coli* heat-shock response; *Mol. Microbiol.* **9** 671–680

Bullen J J 1973 The significance of iron in infection; *Rev. Infect. Dis.* **3** 1127–1138

Calderwood S B and Mekalanos J J 1987 Iron regulation of Shiga like toxin expression in *E. coli* is mediated by the *fur* locus; *J. Bacteriol.* **169** 4759–4764

Calderwood S B and Mekalanos J J 1988 Confirmation of the Fur operator site by insertion of a synthetic oligonucleotide into an operon fusion plasmid; *J. Bacterio1*.**170** 1015–1017

Camilli A, Beattie D T and Mekalanos J J 1994 Use of genetic recombination as a reporter of gene expression; *Proc. Natl. Acad. Sci. USA* **91** 2634–2638

Carreiro M M, Laux D C and Nelson D R 1990 Characterization of the heat shock response and identification of heat shock protein antigens of *Borrelia burgdorferi; Infect. Immun.* **58** 2186–2191

Chatfield S N, Dorman C J, Hayward C and Dougan G 1991 Role of *omp*R dependent genes in *Salmonella typhimurium* virulence: mutants deficient in both OmpC and OmpF are attenuate *in vivo; Infect. Immun.* **59** 449–452

Chippaux M, Bonnefoy V, Ratouchniak J and Pascal M C 1982 Operon fusion in the nitrate reductase operon and study of the control of *nir*R in *Escherichia coli; Mol. Gen. Genet.* **182** 477–479

Cluss R G and Boothby J T 1990 Thermoregulation of protein synthesis in *Borrelia burgdorferi; Infect. Immun.* **58** 1038–1042

Cornelis G, Shuters C, de Rouvroit C L and Michiels T 1989 Homology between VirF, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherikhia coli* arbinose operon regulator, *J. Bacteriol.* **171** 254–263

Cotter P A, Chepuri V, Gennis R B and Gunsalus R P 1990 Cytochrome *o*(*cyo*ABCDE) and *d*(*cyd*AB) oxidase gene expression in *Escherichia coli* is regulated by oxygen, pH and the *fnr* gene product; *J. Bacteriol.* **172** 6333–6338

Cotter P A and Gunsalus R P 1989 Oxygen, nitrate and molybdenum regulation of *dms*ABC gene expression in *Escherichia coli; J. Bacteriol.* **171** 3817–3823

Cotter P A and Gunsalus R P 1992 Contribution of the *fnr* and *arc*A gene products in coordinate regulation of the cytochrome o (*cyo* ABCDE) and d (*cyd* AB) oxidase genes in *Escherichia coli; FEMS Microbiol. Lett.* **91** 31-36

Craig E 1993 Heat shock proteins: Molecular chaperones of protein biogenesis; *Microbiol. Rev.* **57** 402–414

Craig E and Gross C 1991 Is *hsp*70 the cellular thermometer?; *Trends Biochem. Sci.* **16** 135-140

Deretic V, Dikshit R, Konyessni W M, Chakraborty A M and Misra T K 1989 The *alg*R gene, which regulates mucoidy in *Pseudomonas aeruginosa* belongs to a class of environmentally responsive genes; *J. Bacteriol.* **171** 1278–1283

Deretic V, Mohr C D and Martin D W 1991 Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: Signal transduction and histone like elements in the regulation of bacterial virulence; *Mol. Microbiol.* **5** 1577–1583

Dorman C J 1991 DNA supercoiling and environmental regulation of gene expression in pathogenic bacteria; *Infect. Immun.* **59** 745–749

Dorman C J, Chatfield S, Higgins C F, Hayward C and Dougan G 1989 Characterization of porin and *omp*R mutants of a virulent strain of *Salmonella typhimurium: omp*R mutants are attenuated *in vivo; Infect Immun.* **57** 2136–2140

Erickson J W and Gross C A 1989 Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate σ factor involved in high-temperature gene expression; *Genes Dev.* **3** 1462–1471

Ernst J F, Bennett R L and Rothfield L I 1978 Constitutive expression of the iron enterochelin and ferrichrome uptake system in a mutant strain of *Salmonella typhimurium; J. Bacteriol.* **135** 928–934

Galan J E and Curtiss R III 1990 Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling; *Infect. Immun.* **58** 1879–1885

Garvin L D and Hardies G C 1989 Nucleotide sequence for the *htp*R gene from *Citrobacter freundii; Nucleic Acids Res.* **17** 4889

Goldberg M B, Boyko S A and Calderwood S B 1990a Positive transcriptional regulation of an iron regulated virulence gene of *Vibrio cholerae; Proc. Natl. Acad. Sci. USA* **88** 1125–1129

Goldberg M B, Boyko S A and Calderwood S B 1990b Transcriptional regulation by iron of a *Vibrio cholerae* virulence gene and homology of the gene to the *Escherichia coli* Fur system; *J. Bacteriol.* **172** 6863–6870

Graeme-Cook K A, May G, Bremer E and Higgins C F 1989 Osmotic regulation of porin expression: a role for DNA supercoiling; *Mol. Microbiol.* **3** 1287–1294

Henderson D P and Payne S M 1994 *Vibrio cholerae* iron transport systems: Roles of heme and siderophore iron transport in virulence and identification of a gene associated with multiple iron transport systems; *Infect Immun*. **62** 5120–5125

Hoe N P, Minion F C and Goguen J D 1992 Temperative sensing in *Yersenia pestis*: Regulation of *yop*E transcription by *lcr*F*; J. Bacteriol.* **174** 4275–4286

Isberg R R, Swain A and Falkow S 1988 Analysis of expression and thermoregulation of the *Yersinia pseudotuberculosis inv* gene with hybrid proteins; *Infect. Immun.* **56** 2133–2138

Iuchi S and Lin E C C 1991 Adaptation of *Escherichia coli* to respiratory conditions: Regulation of gene expression; *Cell* **66** 5–7

Jones B D and Falkow S 1994 Identification and characterization of a *Salmonella typhimurium* oxygen regulated gene required for bacterial internalization; *Infect. Immun.* **62** 3745–3752

Jones H M and Gunsalus R P 1987 Regulation of *Escherichia coli* fumarate reductase (*frd*ABCD) operon expression by respiratory electron acceptors and the *fur* gene product; *J. Bacteriol.* **169** 3340–3349

Kaufman S H E, Schoel B, Embdes J D A V, Koga T, Wand-Wurttinberger A, Munk M E and Steinhuff U 1991 Heat shock protein 60: Implications for pathogenesis of and protection against bacterial infections: *Immun. Rev*. **121** 67–90

Lambden P R and Guest J R 1976 Mutants of *Escherichia coli* K12 unable to use fumarate as an anaerboic electron acceptor; *J. Gen. Microbiol.* **97** 145–160

Landick R, Vaughn V, Lau E T, Van Bogelen R A, Erickson J W and Neidhart F C 1984 Nucleotide sequence of the heat shock regulatory gene of *E. coli* suggests its protein product may be a transcription factor; *Cell* **38** 175–182

Litwin C M, Boyko S A and Calderwood S B 1992 Cloning, sequencing and transcriptional regulation of the *Vibrio cholerae fur* gene; *J. Bacteriol.* **174** 1897–1903

Looney R J and Steigbigel R J 1986 Role of the Vi antigen of *Salmonella typhimurium* in resistance to host defense *in vitro; J. Lab. Clin. Med.* **108** 506–516

Maurelli A T 1989 Temperature regulation of virulence genes is pathogenic bacteria: a general strategy for human pathogens; *Microb. Pathog.* **7** 1–10

Miller S I and Mekalanos J J 1990 Constitutive expression of the *pho*P regulon attenuates *Salmonella virulence* and survival within macrophages; *J. Bacteriol.* **172** 2485–2490

Miller V L and Mekalanos J J 1988 A novel suicide vector and its use in construction of insertion mutations: Osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *tox*R*; J. Bacteriol.* **170** 2575–2583

Miller V L, Taylor R K and Mekalanos J J 1987 Cholera toxin transcriptional activator ToxR is a transmembrane DNA binding protein; *Cell* **48** 271–279

Murray P G and Young R A 1992 Stress and immunological recognition in host pathogen interactions; *J. Bacteriol.* **174** 4193–4196

Nakahigashi K, Yanagi H and Yura T 1995 Isolation and sequence analysis of *rpo*H genes encoding σ^{32} homologs from gram negative bacteria: conserved mRNA protein segments for heat shock regulation; *Nucleic Acid. Res.* **23** 4383–4390

Parkinson J S and Kofoid E C 1992 Communication modules in bacterial signaling proteins; *Annu. Rev. Genet.* **26** 71–112

Parsot C and Mekalanos J J 1990 Expression of ToxR, the transcriptional activator of the virulence factors in *Vibrio cholerae,* is modulated by the heat shock response; *Proc. Natl. Acad. Sci. USA* **87** 9898–9902

Pickard D, Li J, Roberts M, Maskell D, Hone D, Levine M, Dougan G and Chatfield S 1994 Characterisation of defined *omp*R mutants of *Salmonella typhimurium: omp*R is involved in the regulation of Vi poly saccharide expression; *Infect. Immun.* **62** 3984–3993

Pierson D E and Falkow S 1990 Nonpathogenic isolates of *Yersinia enterocolitica* do not contain functional *inv*-homologous sequences; *Infect. Immun.* **58** 1059–1064

Poole K and Braun V 1988 Iron regulation of *Serratia marcescens* hemolysin gene expression; *Infect. Immun.* **56** 2967–2971

Raina S, Missiakas D and Georopoulos C 1995 The *rpo*E gene encoding the $\sigma^{E}(\sigma^{24})$ heat sock sigma factor of *Escherichia coli; EMBO J.* **14** 1043–1055

Retzlaff C, Yamamoto Y, Hoffman P S, Friedman H and Klein T W 1994 Bacterial heat shock proteins directly induce cytokine mRNA and interleukin I secretion in macrophage cultures; *Infect. Immun.* **62** 5689–5693

Rouviere P E, Las Penas A D, Mecsas J, Lu C Z, Rudd K E and Gross C A 1995 *rpo*E, the gene encoding the second heat shock sigma factor, σ^{E}, in *Escherichia coli; EMBO J.* **14** 1032–1042

Roy N K, Das G, Balganesh T S, Dey S N, Ghosh R K and Das J 1982 Enterotoxin production, DNA repair and alkaline phosphatase of *Vibrio cholerae* before and after animal passage; *J. Gen. Microbiol.* **128** 1927–1932

Sahu G K, Chowdhury R and Das J 1994 Heat shock response and heat shock protein antigens of *Vibrio cholerae; Infect. Immun.* **62** 5624–5631

Schaffer S, Hantke Kand Braun V 1985 Nucleotide sequence of the iron regulatory gene *fur; Mol. Gen. Genet* **200** 110–113

Shinnick T M 1991 Heat shock proteins as antigens of bacterial and parasitic pathogens; *Curr, Top, Microbiol. lmmunol.* **167** 145

Silva C L and Lowrie D B 1994 A single mycobacterial proteins (*hsp*65) expressed by a transgenic antigen presenting cell vaccinates mice against tuberculosis; *Immunology* **82** 244–248

Sokolovic Z, Fuchs A and Goebel W 1990 Synthesis of species specific stress proteins by virulent strains of *Listera monocytogens; Infect. Immun.* **58** 3583–3587

Staggs T M and Perry R D 1991 Complementation of the *Escherichia coli* Fur phenotype by *Yersinia pestis fur,* H-36, *Abstr. 91st Gen. Meet. Am. Soc. Microbiol,* p 161

Stewart V 1982 Requirement of Fnr and NarL functions for nitrate reductase expression in *Escherichia coli* K-12; *J. Bacteriol.* **151** 1320–1325

Stoebner J A and Payne S M 1988 Iron regulated hemolysin production and utilisation of heme and hemoglobin by *Vibrio cholerae; Infect Immun.* **56** 2891–2895

Strauss E J 1995 When a turn off is a turn on; *Curr. Biol.* **5** 706–709

Tobe T, Nagai S, Okada N, Adler B, Yoshikawa M and Sasakawa C 1991 Temperature regulated expression of invasion genes in *Shigella flexneri* is controlled through the transcriptional activation of the *vir*6 gene on the large plasmid; *Mol. Microbiol,* **5** 887–893

Tomoyasu T, Gamer Bukau B, Kanemori M, Mori H, Rutman A J, Oppenheim A B, Yura T, Yamanaka K, Niki Hiraga S and Ogura T 1995 *E, coli* FtsH is a membrane bound ATP-dependent protease which degrades the heat shock transcription factor, σ^{32}; *EMBO J.* **14** 2551–2560

Wachter M L, Domann E and Chakraborty T 1992 The expression of virulence genes in *Listeria monocytogenes* is thermoregulated; *J. Bacteriol.* **174** 947–952

Young D B and Garbe T R 1991 Heat shock proteins and antigens of *Mycobacterium tuberculosis; Infect. Immun.* **59** 3086–3093

Yura T, Nagai H and Mori H 1993 Regulation of the heat shock response in bacteria; *Annu. Rev. Microbiol.* **47** 321–350