

Conserved function and regulation of σ^{32} homologues in Gram-negative bacteria

K NAKAHIGASHI, M KANEMORI, M MORITA, H YANAGI and T YURA*

HSP Research Institute, Kyoto Research Park, Kyoto 600, Japan

*Corresponding author (Fax, 81-75-315-8659; Email, tyura@hsp.co.jp).

The heat shock response in *Escherichia coli* and related bacteria is primarily mediated by σ^{32} or its homologue (RpoH protein) specifically required for transcription of heat shock genes encoding molecular chaperones and proteases. Extensive work in *E. coli* revealed some of the mechanisms controlling the cellular level and activity of σ^{32} during the heat shock response. Recent isolation of a number of RpoH homologues from γ , β and α proteobacteria provided an opportunity to examine evolutionary conservation and diversity of regulatory mechanisms in these bacteria. We here summarize the present status of this aspect of the stress response not only by comparative sequence analysis but by examining the response of representative RpoH homologues of the γ subgroup to heat shock stress. Current evidence indicates that the basic strategy of enhancing RpoH level as a primary response to heat shock stress is well conserved, but the detailed mechanisms for enhancement of the heat shock σ factor level vary among different species that may reflect diverse ecological niches.

1. Introduction

The RpoH gene encoding the heat shock transcription factor σ^{32} plays a central role in regulation of the heat shock response in *E. coli* (Gross 1996; Yura 1996). RNA polymerase holoenzyme containing σ^{32} specifically recognizes heat shock promoters and transcribes genes that encode molecular chaperones and ATP-dependent proteases (Georgopoulos *et al* 1994; Gross 1996). The cellular level of σ^{32} is very low under nonstressed conditions, but is rapidly and transiently enhanced upon exposure to heat or other stresses (Gross *et al* 1990; Bukau 1993; Yura *et al* 1993). The increase in σ^{32} level results from both increased synthesis at the level of translation and stabilization of the otherwise very unstable σ^{32} (Grossman *et al* 1987; Straus *et al* 1987). Extensive work during past years led to identification of several regions on the *rpoH* mRNA or σ^{32} protein that may be critically involved in regulating synthesis and/or stability of σ^{32} (Nagai *et al* 1991, 1994; Yuzawa *et al* 1993). Besides, transacting factors such as DnaK, DnaJ and GrpE chaperones are known to negatively modulate each

of these processes, whereas another heat shock σ factor, σ^E , activates *rpoH* transcription upon exposure to extreme heat stress (Georgopoulos *et al* 1994; Gross 1996).

Meanwhile, the *rpoH* homologues have been cloned from a number of Gram-negative proteobacteria (γ and α subgroups) (see Nakahigashi *et al* 1995; Yura 1996) and their roles in the heat shock response were analysed in some cases. However, very little is known about how the homologues themselves respond to heat shock. We shall summarize current status of this area of research contributed from our and several other laboratories.

2. Distribution of RpoH (σ^{32})-homologues in eubacteria

The first homologue of σ^{32} reported was that from *Citrobacter freundii* which is very closely related to *E. coli*. A number of additional homologues were recently isolated from diverse Gram-negative proteobacteria (Nakahigashi *et al* 1995 and references therein) and the list now includes 18 RpoH homologues from 16 bacterial

Keywords. Heat shock response; heat shock promoter; σ factor; σ^{32} ; *rpoH*; transcription factor

species (figure 1). Many of them were isolated by functional complementation of defects in the *E. coli* Δ *rpoH* mutant that can grow only at or below 20°C and cannot support growth of λ phage (Benvenisti et al 1995; Nakahigashi et al 1995; Narberhaus et al 1996).

Altogether 9 homologues including that of *E. coli* were obtained from γ subgroup, 7 from α subgroup, 1 from β (*Alcaligenes xylosoxydans*; AXY), and 1 from unknown subgroup (*Pseudomonas hydrogenothermophila*; PHY); the latter two are most recent additions to the list

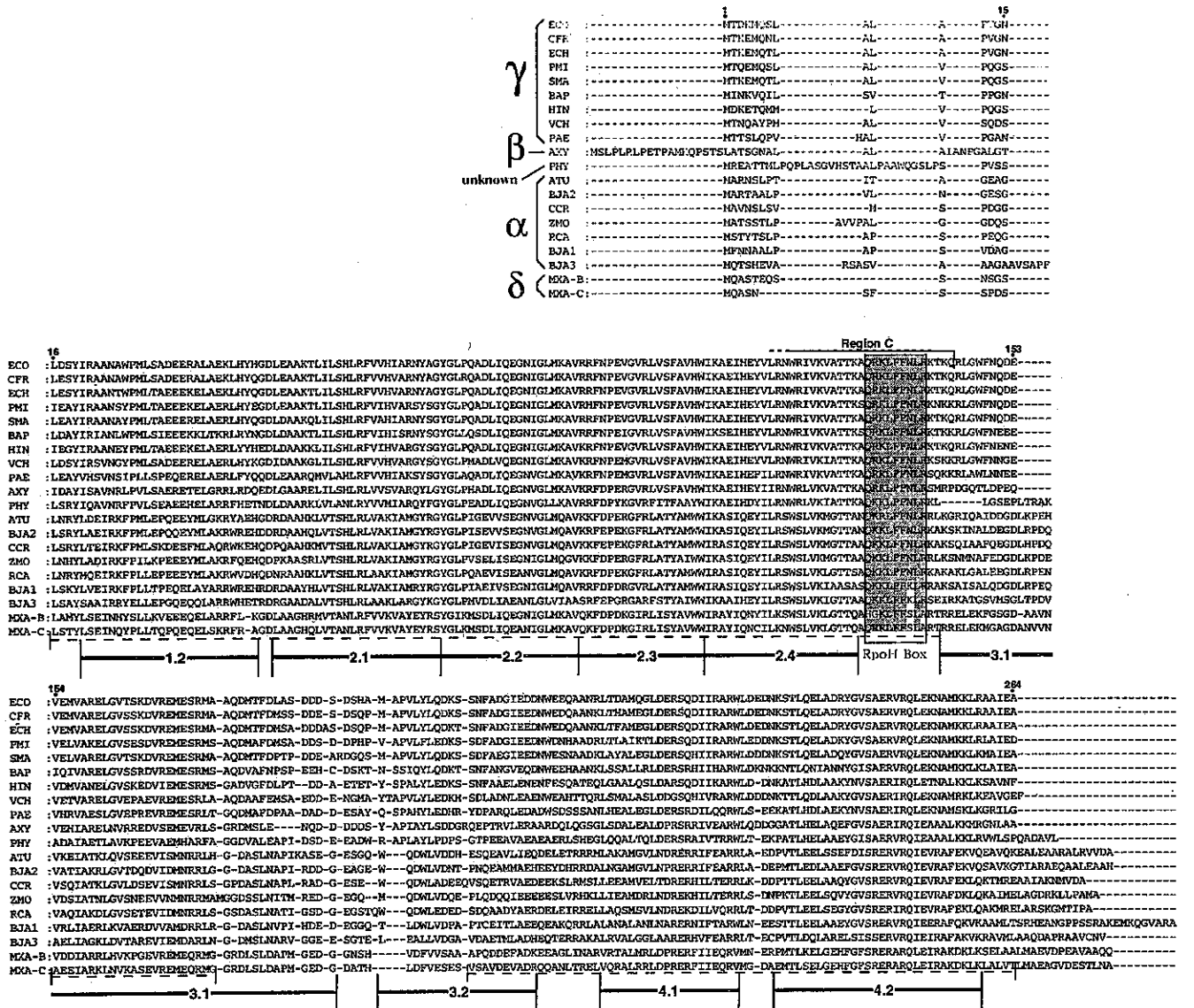


Figure 1. Multiple alignment of deduced amino acid sequences of known RpoH homologues. All sequence data except for those of *A. xylosoxydans* ATCC27061 (AXY) and *P. hydrogenothermophila* IFO14978 (PHY) (Nakahigashi N, unpublished) were obtained from protein databases or deduced from DNA databases: *E. coli* (ECO, A94012); *C. freundii* (CFR, S04697); *E. cloacae* (ECL, D50829); *Proteus mirabilis* (PMI, D50830); *Serratia marcescens* (SMA, D50831); *Buchnera aphidicola* (BAP, U35400); *Haemophilus influenzae* (HIN, P44404); *Vibrio cholerae* (VCH, U44432); *Pseudomonas aeruginosa* (PAE, U09560); *Agrobacterium tumefaciens* (ATU, D50828); *Bradyrhizobium japonicum* (BJA1, U55047; BJA2, Y09502; BJA3, Y09666); *Caulobacter crescentum* (CCR, U37792); *Zymomonas mobilis* (ZMO, D50832); *Rhodobacter capsulatus* (RCA, AF017436). SigB and SigC (MXA-B, X55500; MXA-C, L12992) of *Myxococcus xanthus* are included here for comparison, though they probably do not represent functional RpoH homologues. Multiple alignment was carried out with ICOT Free Software (Ishikawa et al 1994). Numbers below sequences represent generally conserved regions (shown by solid lines) for σ factors (Lonetto et al 1992), and RpoH box (Nakahigashi et al 1995) region C (Nagai et al 1994) are also indicated. Dotted lines show the regions used for phylogenetic analysis (see figure 2).

(Nakahigashi K, unpublished; accession numbers AB009990 and AB009991). Besides, two σ factors of *Mycococcus xanthus* (MXA), SigB and SigC, are related in sequence to the RpoH homologues (figure 1), though their functions are probably not related to the heat shock response.

3. Structural similarity of RpoH homologues

The overall similarity was first examined by aligning the deduced amino acid sequences of each of the RpoH homologues with the representative homologues from γ (*E. coli*; ECO), β (*A. xylosoxydans*; AXY), and α (*A. tumefaciens*; ATU) subgroups (table 1). Thus, the 9 homologues of γ subgroup exhibited amino acid identity of more than 60% with that of ECO (γ), 45 to 49% identity with that of AXY (β), and 34 to 37% identity with that of ATU (α). Similarly, the 5 homologues of α subgroup showed over 60% identity with that of ATU (α), 35 to 37% identity with AXY (β), and 36–39% identity with ECO (γ). These results indicate that the RpoH homologues of γ or α subgroup form distinct clusters, while that of β (AXY) appeared to be more closely related to ECO (γ) than to ATU (α).

Multiple sequence alignment of all these homologues clearly show high similarity particularly for the regions 2.1 to 2.4 and 4.1 to 4.2, known to be highly conserved among all σ factors (Lonetto *et al* 1992) (figure 1). Most strikingly, a stretch of 9 amino acids [Q(R/K)(K/R)LFFNLR] located between the two conserved regions 2.4 and 3.1 is extremely well conserved among the RpoH homologues but not among other σ factors such as σ^{70} (Nakahigashi *et al* 1995). This region, called RpoH box, is found within region C implicated for the negative feedback control of the heat shock response (Nagai *et al* 1994). Interestingly, one of the 3 RpoH homologues recently isolated from root nodule bacteria (*Bradyrhizobium japonicum*) that is essential for growth (BJA2; see figure 1) and is encoded by *rpoH₂* has a perfect RpoH box, whereas a single mismatch is found in the other homologues (BJA1 and BJA3) encoded by

rpoH₁ and *rpoH₃*, respectively, that are not essential (Narberhaus *et al* 1997).

All the 7 RpoH proteins from the α subgroup reported so far are unique in possessing extra 5 amino acids in region 3.1, downstream of the RpoH box; this sequence is not found among the γ homologues (figure 1). The RpoH homologue from *A. xylosoxydans* (AXY; β) is similar to the γ for this region, whereas that from *P. hydrothermophila* (PXY) resembles none of the other homologues.

4. Phylogenetic relationships

To further examine genetic distances among the RpoH homologues, a phylogenetic tree was constructed on the basis of sequence comparison for most regions (dotted lines in figure 1) but omitting those that contain extensive additions or deletions. As expected, RpoH proteins from γ or α subgroup form distinct clusters (figure 2), and the relationships within γ or within α generally appeared to agree with the known genetic distances based on the comparison of 16S rRNA (Woese 1987) or β subunit of RNA polymerase (Mollet *et al* 1996). Evidently, the homologue from β (AXY) is more closely related to γ than to α , whereas that from *Pseudomonas hydrothermophila* (PHY) is not related to any other homologues, in agreement with the comparison of 3.1 region (see above). Interestingly, BJA2, which has a perfect RpoH box and is essential for growth, is most closely related to other RpoH proteins of α as compared to the other two homologues from *B. japonicum*.

5. Functional conservation of RpoH homologues

All the RpoH homologues tested except for that from *B. aphidicola* (symbiont of aphids) can complement growth defects of the $\Delta rpoH$ mutant of *E. coli* at higher temperatures (30–42°C) or λ phage growth, indicating that they can be expressed in *E. coli* and can replace function of σ^{32} at least partially. The conserved catalytic

Table 1. Overall structural similarity of the known RpoH homologues^a.

Subgroup	No. of RpoH homologues known	Size (amino acid residues)	% Identity with RpoH from		
			ECO (γ)	AXY (β)	ATU (α)
γ	9	281–286	> 60.7	45.3–48.9	34.2–36.8
β	1	306	47.6	100	35.3
α	5	295–302	35.6–38.7	34.6–37.1	> 59.9
	(7) ^b	(295–308)	(32.8–38.7)	(34.0–37.1)	(> 46.3)

^aThe RpoH homologue from *Pseudomonas hydrothermophila* was excluded from this list. ECO, *E. coli*; AXY, *Alcaligenes xylosoxydans*; ATU, *Agrobacterium tumefaciens*. ^bNumbers and values in parentheses indicate those when data for BJA1 and BJA3 are included.

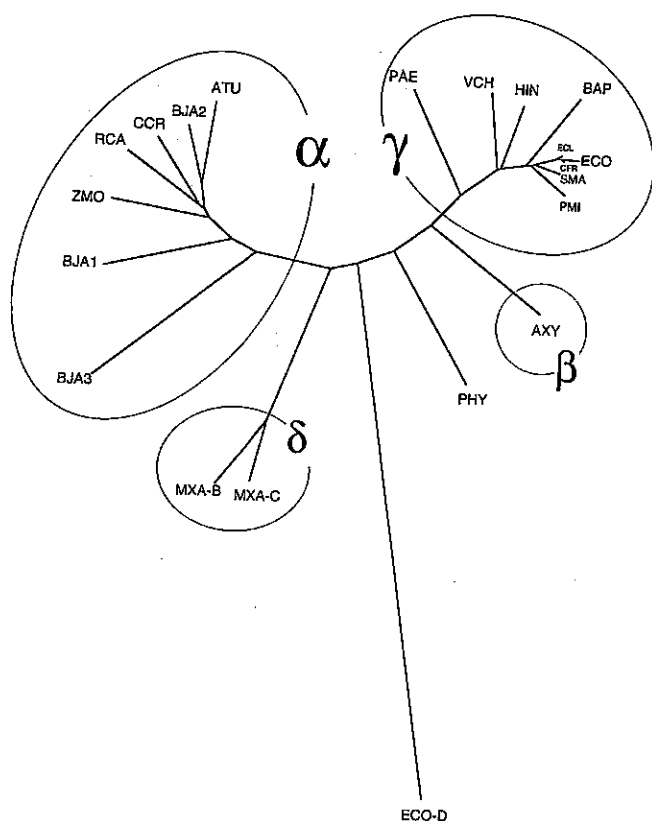


Figure 2. Phylogenetic tree of RpoH homologues. Multiple alignment of RpoH homologues and *E. coli* σ^{70} (ECO-D) was done with ICOT Free Software, and portions of the alignments (dotted lines in figure 1) were analysed sequentially with PROTDIST and FITCH softwares in the PHYLIP package (Felsenstein 1991). The resulting tree was visualized with DRAWTREE in the PHYLIP package. Line segment lengths represent genetic distances based on pairwise comparisons of amino acid sequences.

function of RpoH homologues was confirmed for a variety of homologues by examining *lacZ* expression from *E. coli* heat shock promoters (e.g., Benvenisti et al 1995; Nakahigashi et al 1995). Furthermore, all the homologues tested from γ or α subgroup, when expressed in *E. coli*, were found to recognize *dnaK* and *groE* heat shock promoters and initiate transcription from start sites known to be utilized by *E. coli* σ^{32} , indicating strict conservation in promoter specificity (Nakahigashi et al 1998). Specific recognition of cognate heat shock promoters by purified RNA polymerase containing RpoH was directly verified in the case of *C. crescentus* (Wu and Newton 1996, 1997), suggesting that *E. coli* and *C. crescentus* σ^{32} homologues recognize very similar, if not identical, promoter sequences.

6. Conservation of *rpoH* regions known to be involved in *E. coli* heat shock regulation

The increased level of σ^{32} observed upon heat shock results from both increased synthesis and stabilization. The increased synthesis occurs by activating translation of *rpoH* mRNA whose secondary structure normally restricts translation initiation (Nagai et al 1991; Yuzawa et al 1993; Morita M, unpublished). The stable mRNA secondary structure (figure 3D) is formed between the 15-bp 'region A' immediately downstream of initiation codon and a stretch of internal 'region B' (figure 3B). On the other hand, the stability of σ^{32} seems to involve a segment of σ^{32} (region C; Nagai et al 1994) which contains the RpoH box (see figure 1). The *in vivo* turnover of σ^{32} is modulated by the DnaK-DnaJ-GrpE chaperone team (Straus et al 1990; Tilly et al 1989), and region C is likely to provide site(s) for interaction with DnaK (McCarty et al 1996). The membrane-associated metalloprotease, FtsH (HflB), is critically involved in degradation of σ^{32} (Herman et al 1995; Tomoyasu et al 1995), although recent evidence suggests synergistic roles of several cytosolic ATP-dependent proteases as well in modulating turnover of σ^{32} *in vivo* (Kanemori et al 1997). Besides, more extreme heat shock (e.g., exposure to 45–50°C) activates *rpoH* transcription, since one of the *rpoH* promoters is recognized by σ^E whose activity reflects states of protein folding in extracytoplasmic compartments (Gross 1996).

Table 2 summarizes distribution of the above set of regulatory sequences among the known RpoH homologues. The RpoH box and region C are conserved among all homologues (figure 1), suggesting that the chaperone-mediated feedback control of the heat shock response may be widely conserved not only in γ but also in other subgroups. In contrast, the characteristic mRNA secondary structure is conserved among most members of γ subgroup but not β or α , suggesting that the type of translational control found in *E. coli* may be restricted to γ subgroup. As to transcriptional control of *rpoH*, the σ^E -type promoter appears to be found in most γ homologues suggesting strong conservation, whereas the σ^{32} -type promoter is found in some homologues of α but not the others. The latter issue awaits further experimental verification.

7. Heat induction mechanisms of RpoH in γ subgroup

When cells of three representative γ proteobacteria (*Serratia marcescens*, *P. mirabilis* and *P. aeruginosa*) were grown at 30°C and shifted to 42°C, the RpoH levels as determined by immunoblotting with antisera against *E. coli* σ^{32} increased markedly and transiently much like in *E. coli*. Pulse-labelling experiments with [³⁵S]methionine showed that the synthesis of RpoH is

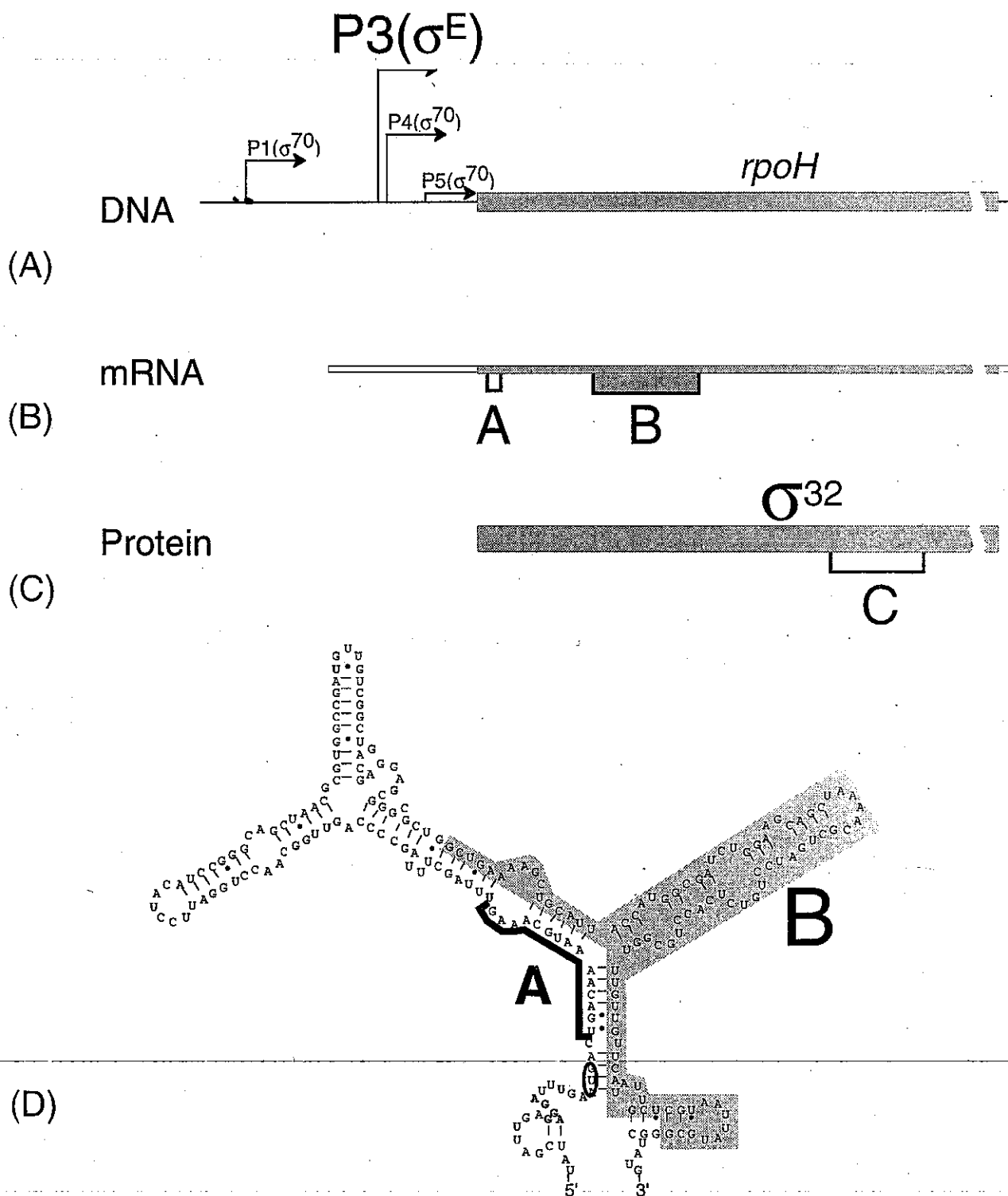


Figure 3. The known regulatory regions of *E. coli* *rpoH* or σ^{32} involved in the heat shock response. (A), (B) and (C) represent schematic diagrams of 5'- or N-terminal regions of the *rpoH* (gene or mRNA) or σ^{32} protein, respectively (Yura *et al* 1993). (A) Among the four known promoters (P1, P3-5), P3 is recognized by σ^E which is activated at 45–50°C, whereas the others are recognized by σ^{70} . (B) Regions A (6–20 nt from the initiation codon) and B (110–210 nt) on *rpoH* mRNA are involved in translational control of σ^{32} synthesis during the heat shock response. (C) Region C (around 122–144 aa) is thought to be critical for the DnaK/J-mediated negative feedback control of the heat shock response. (D) Secondary structure of *rpoH* mRNA region (–20 to +230 nt) including regions A (thick line) and B (shade). The initiation codon (AUG) is encircled.

Table 2. Putative regulatory sequences found in *rpoH* homologues.

<i>rpoH</i>	RpoH box ^a	mRNA secondary structure ^b	Promoters ^c	
			σ^E -type	σ^{32} -type
<i>(\gamma</i> subgroup)				
ECO	+	+	+(exp) ^d	-(exp) ^d
CFR	+	+	+(seq)	-(seq)
ECL	+	+	+(seq)	-(seq)
PMI	+	+	+(seq)	-(seq)
SMA	+	+	+(seq)	-(seq)
BAP	+	-	-(exp) ^e	-(exp) ^e
HIN	+	-	+(seq)	-(seq)
VCH	+	-	NA ^f	NA ^f
PAE	+	+	+(exp) ^g	-(exp) ^g
<i>(\beta</i> subgroup)				
AXY	+	-	-(seq)	-(seq)
<i>(\alpha</i> subgroup)				
ATU	+	-	-(exp) ^h	+(exp) ^h
CCR	+	-	-(exp) ⁱ	+(exp) ⁱ
BJA2	+	-	+(exp) ^j	-(exp) ^j
ZMO	+	-	-(seq)	-(seq)
RCA	+	-	-(seq)	+(seq)
BJA1	+/-	-	-(exp) ^k	-(exp) ^k
BJA3	+/-	-	-(exp) ^l	+(exp) ^l

^a+, perfect matching with consensus; +/-, a single mismatching.

^bPresence (+) or absence (-) of stable mRNA secondary structure predicted for region (-20 to +230 nt) similar to that of *E. coli rpoH*. ^cPresence (+) or absence (-) of respective promoter based on experimental evidence (exp) or deduced from sequence data (seq). ^dGross et al (1990). ^eSato and Ishikawa (1997). ^fNo sequence information available. ^gNaczinski et al (1995). ^hNakahigashi K (unpublished). ⁱReisenauer et al (1996) and Wu and Newton (1996). ^jNarberhaus et al (1997). ^kNarberhaus et al (1996).

transiently induced, although the kinetics and the extent of heat induction varied in different species (figure 4A). Similar heat induction occurred even when cells were pretreated with rifampicin, suggesting that the induction takes place at the level of translation, as found in *E. coli*. However, such heat induction at the translational level may not be found in all γ proteobacteria, in view of the variation in predicted *rpoH* mRNA secondary structures (e.g., Sahu et al 1997).

Stabilization of RpoH homologues upon heat shock was observed with 2 of the 3 species tested (*S. marcescens* and *P. aeruginosa*) but not with *P. mirabilis* whose RpoH protein was very stable even during steady-state growth with little further stabilization upon heat shock (figure 4B). When the latter RpoH (PMI) was produced in *E. coli*, however, it was quite unstable like *E. coli* σ^{32} , but was stabilized upon temperature upshift (data not shown). These results suggest that the apparent stability of RpoH (PMI) may not be due to intrinsic stability of the protein but rather to the nature of proteolytic system of *P. mirabilis* (Nakahigashi et al 1998).

8. Regulatory mechanisms of RpoH in α and β subgroups

The apparent lack of conserved mRNA secondary structure among RpoH homologues of α or β subgroup suggested that unlike in *E. coli* the translational control of RpoH induction in these subgroups may not occur as part of the heat shock regulatory mechanism. Recent results with RpoH of *C. crescentus* (Reisenauer et al 1996; Wu and Newton 1996) and *B. japonicum* (Narberhaus et al 1997) revealed that heat induction occurs at the level of transcription. Interestingly, one of the promoters for *C. crescentus rpoH* is heat inducible and is recognized by RpoH itself, as directly demonstrated by *in vitro* experiments (Wu and Newton 1997). In *B. japonicum*, the heat-inducible promoter for *rpoH*₂ resembles the σ^E -type promoter, whereas that of *rpoH*₃ is similar to the σ^{32} consensus (table 2). The positive autogenous regulation of RpoH in *C. crescentus* could be counteracted by a separate negative control mechanism, as has recently been found for a second heat shock σ factor, σ^E in *E. coli* (Missiakas et al 1997; Penas et al 1997).

Although stabilization may not play a major role in regulation of these RpoH homologues during heat shock induction, the presence of highly conserved RpoH box and region C suggests that the chaperone-mediated control of RpoH turnover might be conserved among α proteobacteria as well. The control of RpoH activity as observed in *E. coli* under conditions of excess heat shock proteins (Straus et al 1989; Taura et al 1989) would be another potentially important aspect of regulation for future investigation.

9. Conclusions and perspectives

Genes homologues to *E. coli rpoH* have so far been isolated from γ , β and α proteobacteria but not from other bacteria including δ or ϵ proteobacteria. Although the present list may expand further, its distribution may well be restricted within proteobacteria. This would imply that the heat shock regulatory mechanism mediated by σ^{32} (and its homologues) evolved after separation of proteobacteria from other Gram-negative bacteria. Evidence suggests that all the bacteria tested that are known to have RpoH homologues respond to heat shock stress by enhancing the cellular level of RpoH, which leads to induction of heat shock proteins to meet with increased cellular requirement for molecular chaperones and energy-dependent proteases.

As to the details of regulatory strategies employed, many of the γ proteobacteria seem to utilize multiple network affecting translation and stability of RpoH that brings about rapid and transient enhancement of σ^{32} homologue as has been unravelled in *E. coli*. In contrast,

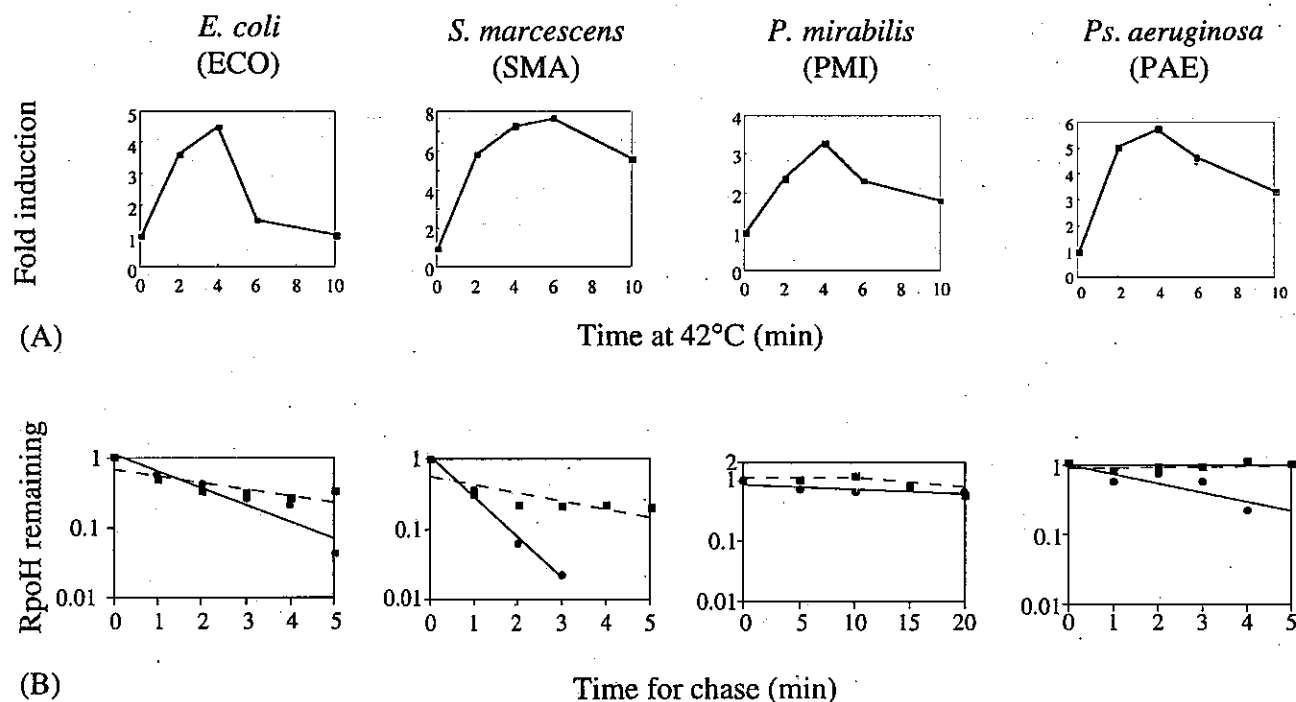


Figure 4. Effects of temperature upshift (30 to 42°C) on the synthesis and stability of RpoH homologues from γ proteobacteria. (A) Synthesis rates of RpoH as determined by pulse-labelling with [35 S]methionine upon shift to 42°C followed by immunoprecipitation and SDS-PAGE. (B) Pulse-chase experiments examining stability of RpoH during steady-state growth at 30°C (solid lines) or after shift to 42°C (dotted lines).

α proteobacteria apparently utilize distinct strategies including transcriptional activation by RpoH itself. Further analyses particularly with the latter system should reveal new insights into function and regulation as well as evolutionary conservation and diversity of σ^{32} homologues.

Acknowledgements

We thank Dr M Ishikawa for use of computer program and Ms Masako Nakayama, Mayumi Ueda, and Hideaki Kanazawa for technical assistance.

References

- Benvenisti L, Koby S, Rutman A, Giladi H, Yura T and Oppenheim A B 1995 Cloning and primary sequence of the *rpoH* gene from *Pseudomonas aeruginosa*; *Gene* **155** 73–76
- Bukau B 1993 Regulation of the *Escherichia coli* heat-shock response; *Mol. Microbiol.* **9** 671–680
- Felsenstein J 1991 *PHYLIP manual*, version 3.5, University of Washington, Seattle, WA, USA
- Georgopoulos C, Liberek K, Zylicz M and Ang D 1994 Properties of the heat shock proteins of *Escherichia coli* and the autoregulation of the heat shock response; in *The biology of heat shock proteins and molecular chaperones* (eds R I Morimoto, A Tissieres and C Georgopoulos (New York: Cold Spring Harbor Laboratory Press) pp 209–249
- Gross C A 1996 Function and regulation of the heat shock proteins; in *Escherichia coli and Salmonella: cellular and molecular biology* (eds F C Neidhardt, R Curtiss III, J L Ingraham, E C C Lin, K B Low, B Magasanik, W S Reznikoff, M Riley, M Schaechter and H E Umbarger (Washington, DC: ASM Press) pp 1382–1399
- Gross C A, Straus D B, Erickson J W and Yura T 1990 The function and regulation of heat shock proteins in *Escherichia coli*; in *Stress proteins in biology and medicine* (eds R I Morimoto, A Tissieres and C Georgopoulos (New York: Cold Spring Harbor Laboratory Press) pp 167–189
- Grossman A D, Straus D B, Walter W A and Gross C A 1987 σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*; *Genes Dev.* **1** 179–184
- Herman C, Thevenet D, D'Ari R and Boulloc P 1995 Degradation of σ^{32} , the heat shock regulator in *Escherichia coli* is governed by *hflB*; *Proc. Natl. Acad. Sci. USA* **92** 3516–3520
- Ishikawa M, Toya T and Totoki Y 1994 *Proceedings of the International Symposium on Fifth Generation Computer Systems*, pp 129–138
- Kanemori M, Nishihara K, Yanagi H and Yura T 1997 Synergistic roles of HsIVU and other ATP-dependent proteases in controlling in vivo turnover of σ^{32} and abnormal proteins in *Escherichia coli*; *J. Bacteriol.* **179** 7219–7225
- Lonetto M, Gribskov M and Gross C A 1992 The σ^{70} family: sequence conservation and evolutionary relationships; *J. Bacteriol.* **174** 3843–3849
- McCarty J S, Rudiger S, Schonfeld H-J, Schneider-Mergener J, Nakahigashi K, Yura T and Bukau B 1996 Regulatory region C of the *E. coli* heat shock transcription factor, σ^{32} , constitutes

- a DnaK binding site and is conserved among eubacteria; *J. Mol. Biol.* **256** 829–837
- Missiakas D, Mayer M P, Lemaire M, Georgopoulos C and Raina S 1997 Modulation of the *Escherichia coli* σ^E (RpoE) heat-shock transcription-factor activity by the RseA, RseB and RseC proteins; *Mol. Microbiol.* **24** 355–371
- Mollet C, Drancourt M and Raoult D 1996 *rpoB* sequence analysis as a novel basis for bacterial identification; *Mol. Microbiol.* **26** 1005–1011
- Naczinski Z M, Mueller C and Kropinski A M 1995 Cloning the gene for the heat shock response positive regulator (sigma 32 homolog) from *Pseudomonas aeruginosa*; *Can. J. Microbiol.* **41** 75–87
- Nagai H, Yuzawa H, Kanemori M and Yura T 1994 A distinct segment of the σ^{32} polypeptide is involved in DnaK-mediated negative control of the heat shock response in *Escherichia coli*; *Proc. Natl. Acad. Sci. USA* **91** 10280–10284
- Nagai H, Yuzawa H and Yura T 1991 Interplay of two cis-acting mRNA regions in translational control of σ^{32} synthesis during the heat shock response of *Escherichia coli*; *Proc. Natl. Acad. Sci. USA* **88** 10515–10519
- Nakahigashi K, Yanagi H and Yura T 1995 Isolation and sequence analysis of *rpoH* genes encoding σ^{32} homologs from gram negative bacteria: conserved mRNA and protein segments for heat shock regulation; *Nucleic Acids Res.* **23** 4383–4390
- Narberhaus F, Krummenacher P, Fischer H-M and Hennecke H 1997 Three disparately regulated genes for σ^{32} -like transcription factors in *Bradyrhizobium japonicum*; *Mol. Microbiol.* **24** 93–104
- Narberhaus F, Weiglhofer W, Fischer H M and Hennecke H 1996 The *Bradyrhizobium japonicum rpoH*₁ gene encoding a σ^{32} -like protein is part of a unique heat shock gene cluster together with *groESL*₁ and three small heat shock genes; *J. Bacteriol.* **178** 5337–5346
- Penas A, Connolly L and Gross C A 1997 Two σ^E -mediated response to extracytoplasmic stress in *Escherichia coli* is transduced by RseA and RseB, two negative regulators of σ^E ; *Mol. Microbiol.* **24** 373–385
- Reisenauer A, Mohr C D and Shapiro L 1996 Regulation of a heat shock σ^{32} homolog in *Caulobacter crescentus*; *J. Bacteriol.* **178** 1919–1927
- Sahu G K, Chowdhury R and Das J 1997 The *rpoH* gene encoding σ^{32} homolog of *Vibrio cholerae*; *Gene* **189** 203–207
- Sato S and Ishikawa H 1997 Expression and control of an operon from an intracellular symbiont which is homologs to the *groE* operon; *J. Bacteriol.* **179** 2300–2304
- Straus D B, Walter W A and Gross C A 1987 The heat shock response of *E. coli* is regulated by changes in the concentration of σ^{32} ; *Nature (London)* **329** 349–351
- Straus D B, Walter W A and Gross C A 1989 The activity of σ^{32} is reduced under conditions of excess heat shock protein production in *Escherichia coli*; *Genes Dev.* **3** 2003–2010
- Straus D B, Walter W and Gross C A 1990 DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32} ; *Genes Dev.* **4** 2202–2209
- Taura T, Kusukawa N, Yura T and Ito K 1989 Transient shutoff of *Escherichia coli* heat shock protein synthesis upon temperature shift down; *Biochem. Biophys. Res. Commun.* **163** 438–443
- Tilly K, Spence J and Georgopoulos C 1989 Modulation of stability of the *Escherichia coli* heat shock regulatory factor σ^{32} ; *J. Bacteriol.* **171** 1585–1589
- Tomoyasu T, Gamer J, Bukau B, Kanemori M, Mori H, Rutman A J, Oppenheim A B, Yura T, Yamanaka K, Niki H, Hiraga S and Ogura T 1995 *Escherichia coli* FtsH is a membrane-bound, ATP-dependent zinc-metalloprotease with activity for the heat-shock transcription factor σ^{32} ; *EMBO J.* **14** 2551–2560
- Woese C R 1987 Bacterial evolution; *Microbiol. Rev.* **51** 221–271
- Wu J and Newton A 1996 Isolation, identification, and transcriptional specificity of the heat shock sigma factor σ^{32} from *Caulobacter crescentus*; *J. Bacteriol.* **178** 2094–2101
- Wu J and Newton A 1997 The *Caulobacter* heat shock sigma factor gene *rpoH* is positively autoregulated from a σ^{32} -dependent promoter; *J. Bacteriol.* **179** 514–521
- Yura T, Nagai H and Mori H 1993 Regulation of the heat-shock response in bacteria; *Annu. Rev. Microbiol.* **47** 321–350
- Yura T 1996 Regulation and conservation of the heat-shock transcription factor σ^{32} ; *Genes Cells* **1** 277–284
- Yuzawa H, Nagai H, Mori H and Yura T 1993 Heat induction of σ^{32} synthesis mediated by mRNA secondary structure: a primary step of the heat shock response in *Escherichia coli*; *Nucleic Acids Res.* **21** 5449–5455