Induction of the heat shock response of *E. coli* through stabilization of σ^{32} by the phage λ cIII protein

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The cIII protein of phage λ favors the lysogenic response to infection by inhibiting the degradation of the λ cII protein, which exerts the primary control on the developmental decision for lysis or lysogeny. To study the mechanism and scope of cIII-mediated regulation, we have used plasmid systems to examine the specific effect of cIII overproduction on the growth of *Escherichia coli* and the synthesis of bacterial proteins. We have found that maximal production of cIII prolongs the heat-induced synthesis of *E. coli* heat shock proteins and provokes elevated production of heat shock proteins even at low temperature. The overproduction of heat shock proteins is correlated with a rapid inhibition of cell growth, as judged by measurements of optical density. We suggest that an overactive heat shock response inhibits bacterial growth, either because excessive production of one or more of the proteins is highly deleterious or because only heat shock promoters are transcribed efficiently. To examine the effect of cIII on σ^{32} , the specificity factor for the heat shock response, we have studied the stability of σ^{32} in cells carrying both cIII- and σ^{32} -producing plasmids; the half-life of σ^{32} is increased fourfold in the presence of cIII. We conclude that overproduction of cIII provokes the heat shock response by increasing the steady-state level of active σ^{32} . These studies also support the concept that the rate of expression of heat shock proteins is directly correlated with the amount of active σ^{32} and that regulation of the stability of σ^{32} may be an important factor for control of the heat shock response.

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Bacteriophage λ development depends on a set of phage-specified regulatory proteins that interact with host target proteins to define the temporal pattern of gene expression (Echols 1980; Herskowitz and Hagen 1980; Friedman et al. 1984; Echols 1986). The interplay between viral and host proteins is complex and in many cases undefined in molecular terms (Court and Oppenheim 1983; Friedman et al. 1984). Among several effects on host protein synthesis, phage λ provokes the overproduction of some bacterial proteins induced in the heat shock response; this effect depends on early gene expression from the leftward (p_L) transcription unit of λ (Draho and Hendrix 1982; Kochan and Murialdo 1982).

Production of heat shock proteins is induced shortly after a rapid temperature shift to 42°C, after which synthesis declines to a new steady-state level about twofold greater than that for cells maintained at 30°C (Neidhardt

et al. 1984). Transcription of heat shock genes depends on a promoter-specific subunit of RNA polymerase called σ^{32} (Grossman et al. 1984). The effect of λ on heat shock proteins might derive from a direct action on σ^{32} or a more indirect signal of stress, since other cellular abuse besides heat will turn on the heat shock response (Neidhardt et al. 1984).

One λ -specified regulatory protein from the p_L operon with a potential for host interaction is cIII. The cIII protein favors the lysogenic response to infection by limiting the degradation of λ cII protein, which exerts the primary control on the developmental decision for lysis or lysogeny (Hoyt et al. 1982; Rattray et al. 1984; Banuett et al. 1986; Echols 1986). Because cIII appears to protect cII from more than one protease system (Banuett et al. 1986), cIII might affect the stability of *Escherichia coli* proteins as well. We have found that overproduction

of cIII protein from a plasmid system produces elevated synthesis of heat shock proteins and stabilizes σ^{32} . We conclude that the capacity of λ to induce the synthesis of heat shock proteins probably derives from the ability of cIII to stabilize λ^{32} .

Results

Inhibition of cellular growth by cIII

The precise location of the cIII gene has been determined by DNA sequencing of wild-type and mutant genes, and the gene product has been identified as a 6-kD protein by acrylamide gel electrophoresis (Knight and Echols 1983). To study the mechanism and scope of cIII-mediated regulation, we have used a series of plasmid constructions to examine the specific effect of cIII on the growth of *E. coli* and the synthesis of bacterial proteins. The plasmid constructions are shown in Figure 1 and described in detail in Materials and Methods; plasmids with λ p_L promoter were controlled by a temperature-sensitive cI protein and constructs with a *tac* promoter by the *lac* repressor and IPTG.

The initial indication that cIII interacts strongly with a host regulatory system came from a comparison of bacterial growth after production of cIII from plasmid strains containing the λ genes *N*, *ral*, *ea10*, and cIII, in which the cIII gene was either wild-type or carried a nonsense mutation (cIIIam611) or an overproducing mutation (cIII_{s2}) (Fig. 1a). The cIII_{s2} mutation relieves a translational block to cIII gene expression, while maintaining the wild-type amino acid sequence (D. Knight and H. Echols, unpubl.; Altuvia and Oppenheim 1986). After a temperature shift to 42°C to derepress the p_L plasmid promoter, bacterial growth is severely inhibited for the cIII_{s2} strain (Fig. 2a), but not for cIIIam (Fig. 2b).

Figure 1. Plasmids producing cIII protein. (a) pDK280: The HindIII–SalI fragment of λ with cIII replaced the small HindIII–SalI fragment of pACYC184 (Knight and Echols 1983). The cIII gene was either wild type or carried the cIII_{s2} (overproducing) or cIIIam611 (nonsense) mutations. The cIII gene was expressed from the λ p_L promoter after thermal inactivation of cI temperature-sensitive repressor. (b) pHBA1: An EcoRI–SalI fragment with the cIII gene replaced the small EcoRI–SalI fragment of pKK223-3. The EcoRI–HaeIII fragment (hatched), including the initiation codon of the cIII gene, was chemically synthesized to maximize cIII production (see Materials and methods).

Figure 2. Effect of cIII overproduction on the growth of *E. coli*. Bacteria C600Su[−] carrying derivatives of the plasmid pDK280 were shifted to 42°C at the times indicated (↑), inducing the heat shock response and expression of the λ genes from the p_L promoter. (a) The cIII gene carried the cIII_{s2} (overproducing) mutation; (b) the cIII gene carried the cIIIam611 nonsense mutation.

The wild-type cIII gene also inhibited bacterial growth, though not as severely (data not shown). From these experiments, we conclude that cIII overproduction is highly deleterious to bacterial growth, whereas overexpression of upstream early λ genes is not.

Prolongation of the heat shock response by cIII

To characterize the growth inhibition caused by cIII, we analyzed the pattern of protein synthesis under conditions of cIII overproduction from the p_L plasmids. For this purpose, we pulse-labeled proteins for 1 min with [³⁵S]methionine at different time points before and after the temperature upshift and separated the extracted proteins by acrylamide gel electrophoresis in the presence of SDS. Figure 3 shows a comparison of three strains: no plasmid, the cIIIam611 plasmid, and the cIII_{s2} plasmid. The temperature upshift has two consequences: (1) enhanced synthesis of *E. coli* heat shock proteins and (2) expression of the λ genes from the plasmid (see Fig. 1). The standard heat shock response is shown in Figure 3,

Figure 3. Stabilization of the heat shock response by cIII. Proteins were pulse-labeled with [35 S]methionine and visualized by autoradiography. Indicated on the right side are the migration positions of some heat shock proteins (HS) and the plasmid-encoded λ protein Ea10. Lane 1 contains protein from an uninduced culture. The other lanes contain proteins from cultures that were subjected to a temperature shift from 30°C to 42°C. The bacteria (C600Su $^{-}$) carried either no plasmid or the *cIIIam*611 or *cIIIls*2 plasmids as for Fig. 2. Proteins were labeled for 1 min at different times after the temperature shift (time in minutes indicated by the numbers above each lane), extracted, and fractionated in a 9–19% gradient SDS-polyacrylamide gel.

lanes 1–3; easily identified heat shock proteins are designated HS. Production of the heat shock proteins increases sharply by 5 min and decreases to the steady-state rate by 30 min. A similar pattern is observed for the plasmid carrying the nonsense mutation in the *cIII* gene, except that the λ protein Ea10 appears as a major product (lanes 4–5). In contrast, the overproduction of *cIII* protein by the *cIIIls*2 plasmid maintains the synthesis of heat shock proteins at or close to the transient rate [compare lanes 5 and 7; *cIII* cannot be seen because it comigrates with the major lowest-molecular-weight *E. coli* protein (Knight and Echols 1983)]. The observation that massive overproduction of λ Ea10 does not prolong the heat shock response in the *cIII* $^{-}$ control experiment indicates that the *cIII* effect is not a general consequence of overproduction of “foreign” proteins (Goff and Goldberg 1985). From the experiments of Figure 3, we conclude that the *cIII* protein is involved in an interaction with the heat shock regulatory system of *E. coli*.

Induction of the heat shock response by *cIII*

The experiments presented in Figure 3 do not address the question of whether *cIII* can turn on the heat shock response as well as maintain it. The possibility was also left open that *cIII* might cooperate with other λ proteins encoded by the plasmid. To examine these points, we constructed a plasmid having as its only λ gene an over-producing version of the *cIII* gene expressed from the *tac* promoter under the control of the *lac* repressor (pHBA1 of Fig. 1b). Induction of the *cIII* gene by IPTG inhibited bacterial growth substantially at 30°C and completely at 37°C, indicating that the *cIII*-mediated host interaction could occur at low temperatures (Table 1). The inhibition of growth is correlated with temperature and amount of *cIII* production (Table 1); thus, the growth pattern is consistent with an interaction between *cIII* and the heat shock regulatory system.

To provide an unambiguous and quantitative characterization of the activity of *cIII*, we used two-dimensional gel electrophoresis of 35 S-labeled proteins to compare the normal heat shock response (temperature upshift to 42°C) with the protein pattern elicited by *cIII* overproduction at 30°C (Fig. 4). The typical thermal induction is shown in panels a and b, in which eight heat shock proteins are circled (e.g., Neidhardt et al. 1984). The capacity of *cIII* to induce these same proteins at 30°C is shown in panels d and e. To estimate the relative induction ratios, we used a densitometer to scan the protein spots for the eight heat shock proteins and two proteins (C_a and C_b) for which synthesis was unaffected by temperature, IPTG, or *cIII*. The results of these measurements are given in Table 2. All eight of the heat shock proteins are markedly induced by *cIII* overproduction in the presence of IPTG (the +*cIII* columns $\pm$ IPTG). From the data for Figure 4 and Table 2, we conclude that the *cIII* protein alone can induce the heat shock response.

Stabilization of σ^{32} by *cIII*

The known key control element activating the heat shock response is the product of the *htpR* (*rpoH*) gene

Table 1. Effect of temperature and *cIII* production on bacterial growth

Temperature (°C)	Inhibition of growth (%)	
	high <i>cIII</i>	low <i>cIII</i>
30	40	0
37	100	10
42	100	35

Maximal *cIII* production (high *cIII*) was obtained from the *cIIIsp* gene under the control of the *tac* promoter (pHBA1). Lower *cIII* production (low *cIII*) was provided by the wild-type *cIII* gene under the normal *lac* promoter (pDK520). Bacteria (JM101F' *lacI* Q) were grown in M9 minimal medium. Induction of *cIII* synthesis was achieved in each case by the addition of IPTG. The inhibition was defined by the differential growth rate with or without IPTG.

Figure 4. Induction of the heat shock response by cIII. Proteins were pulse-labeled with [³⁵S]methionine, extracted, fractionated in two-dimensional gels, and visualized by autoradiography. The bacteria (JM101F'*lacI*^Q) carried either the cIII-overproducing plasmid pHBA1 (*d,e*) or the parental (non-cIII) plasmid pKK223.3 (*a, b, c*). Treatment of cells and labeling time were as follows: (*a* and *d*) labeled during exponential growth at 30°C; (*b*) labeled 5 min after temperature shift from 30°C to 42°C; (*c* and *e*) labeled 30 min after addition of IPTG to cells grown at 30°C. Proteins affected by temperature shift or cIII production are circled and identified according to Neidhardt et al. (1984): 1, HtpM; 2, DnaK; 3, HtpG; 4, lysyl-tRNA-synthetase form II; 5, GroEL; 6, HtpI; 7, HtpH; 8, HtpA. C_a and C_b are two proteins unaffected by cIII production, heat shock, or IPTG that were used as controls for the determination of relative synthesis rates (see Table 1).

(Neidhardt and Van Bogelen 1981; Yamamori and Yura 1982; Landick et al. 1984). The product of the *htpR* gene is the RNA polymerase subunit σ^{32} , which directs initiation of transcription to heat shock promoters (Grossman et al. 1984). The σ^{32} protein is unstable (Grossman et al. 1987; K. Tilly and C. Georgopoulos, unpubl.). If cIII acts to increase the stability of σ^{32} as it does for λ cII, cIII might act to turn on the heat shock response through higher steady-state levels of σ^{32} .

To test the possibility of a direct effect of cIII on σ^{32} , we have used a two-plasmid system: the cIII-producing

vector of Figure 1b and a compatible vector carrying the *htpR* gene. Both cIII and σ^{32} were produced from a *tac* promoter in response to IPTG. To measure the stability of σ^{32} , cultures induced at 30°C were pulse-labeled with [³⁵S]methionine, followed by the addition of unlabeled methionine, extraction of protein, and two-dimensional gel electrophoresis. The migration position of σ^{32} was identified by its known molecular weight and isoelectric focusing position (Grossman et al. 1984) (σ^{32} is not made in sufficient quantity in the absence of an overproducing plasmid to be readily seen in two-dimensional gels). The

Table 2. Relative synthesis of heat shock proteins

Gel number	Protein	No cIII present			cIII present	
		standard	T-jump	+IPTG	-IPTG	+IPTG
1	HtpM	1	22	0.9	1	5.8
2	DnaK	1	2.3	0.8	1	2.3
3	HtpG	1	6.3	1.0	1	3.8
4	Lysyl-tRNA-synthetase	1	9.2	2.0	1	3.7
5	GroEL	1	3.1	1.0	1	2.0
6	HtpI	1	5.0	0.9	1	4.3
7	HtpH	1	22	0.9	1	3.7
8	HtpA	1	8.1	0.9	1	4.9
C _a		1	1	1	1	1
C _b		1	0.9	0.9	1	0.9

To derive the relative rates of synthesis for normal heat shock and cIII induction, the densitometer numbers for data columns 1 (no cIII) and 4 (+ cIII) were set as 1. For the other data columns, the densitometer numbers are expressed relative to the control columns (normalized to protein C_a = 1). For the "T-jump" column, a 30°C culture was shifted to 42°C for 5 min prior to labeling. For the IPTG columns, IPTG was added for 30 min prior to labeling. The induction ratios for HtpM and HtpH are subject to considerable error because very little labeled protein was present before induction (see Fig. 4).

Figure 5. Effect of cIII on σ^{32} stability. Synthesis of the σ^{32} and cIII proteins was induced by the addition of IPTG to bacteria carrying the *htpR* and cIII genes on separate plasmids (see Materials and methods). Proteins were pulse-labeled with [35 S]methionine for 1 min at 30°C (15 min after the addition of the inducer IPTG), extracted, and fractionated in two-dimensional gels. After the 35 S-labeling, unlabeled methionine was added, and samples were taken 30 min later to determine the stability of σ^{32} . (Panel 1) Synthesis of σ^{32} in the absence of cIII; (panel 3) synthesis of σ^{32} in the presence of cIII; (panel 2) σ^{32} remaining after 30 min in unlabeled medium; (panel 4) σ^{32} remaining after 30 min in unlabeled medium in the presence of cIII. The σ indicates the position of σ^{32} (Grossman et al. 1984). The protein marked with a diamond ($\diamond$) is a standard stable protein used for the determination of σ^{32} stability (see part b). (b) The relative amount of σ^{32} remaining has been determined from densitometer tracings of gels similar to those in a and plotted as a function of time after the addition of unlabeled methionine. ($\circ$) cIII absent; ($\bullet$) cIII present.

capacity of cIII to stabilize σ^{32} can be clearly seen in the gel photographs of Figure 5a and the quantitative data of Figure 5b. In Figure 5a, the labeled σ^{32} has almost completely disappeared by 30 min in the absence of cIII (panels 1 and 2), whereas about 50% remains in the presence of cIII (panels 3 and 4). The rate of synthesis of σ^{32} is the same with or without cIII, indicating that transcription and translation for *htpR* are not altered (compare panels 1 and 3; additional data not shown). From the quantitative comparison of Figure 5b, we estimate that the half-life of σ^{32} is increased about fourfold in the presence of cIII (from 8 min to 32 min). From the data of Figure 5, we conclude that cIII turns on the heat shock response by stabilizing σ^{32} and thereby increasing the amount of σ^{32} present to direct transcription to heat shock promoters. Although the cIII- σ^{32} interaction is clear, the quantitative half-lives reflect an artificial situation in which both σ^{32} and cIII are overproduced; the half-life of chromosomal-encoded σ^{32} determined by antibody analysis is about 1 min (D.B. Straus, unpubl.).

Discussion

Interaction between cIII and σ^{32}

On the basis of the experiments reported here, we conclude that overproduced λ cIII protein induces the heat shock response by stabilizing σ^{32} , the transcriptional activator for heat shock promoters. This capacity of cIII is similar to the previously defined regulatory activity of cIII for λ development—stabilization of λ cII (Hoyt et al. 1982; Banuett et al. 1986). Thus, cIII clearly has a direct or indirect anti-protease activity. However, the mechanism by which the two proteins are protected from degradation is unclear. The major pathway for degradation of λ cII involves the HflA and HflB proteins (Gautsch and Wulff 1974; Hoyt et al. 1982; Banuett et al. 1986). In contrast, we have found little or no effect of an *hflA*⁻ mutation on the stability of σ^{32} in our plasmid system (H. Bahl, unpubl.); however, a more extensive analysis with *hflA*⁻ and *hflB*⁻ mutants is required before conclusions can be drawn about the role of the Hfl degradation

system. Because cIII protects cII, even in an *hflA⁻ hflB⁻* mutant (Banuett et al. 1986), cIII might protect σ^{32} mainly from an Hfl-independent pathway.

From the limited available information, there are two very different possibilities for the action of cIII: (1) an inhibitor of multiple proteases and (2) a protector of a class of proteins with a common structural feature. The cIII protein is not a completely general protease inhibitor because some unstable *E. coli* proteins are not protected by cIII (H. Bahl, unpubl.); however, cIII might be a multiprotease inhibitor of the Hfl system and one or more other protease systems. The cII and σ^{32} proteins are both transcription regulators that associate with DNA, and so a similarity in structure might lead to a protective interaction with cIII.

Implications for regulation of the heat shock response

There are some interesting implications for heat shock regulation from the physiological consequences of the interaction of cIII and σ^{32} . First, the normal heat shock response is transient—a rapid induction followed by a return to a mildly elevated steady state (see Fig. 3 and Neidhardt et al. 1984). The severe inhibition of bacterial growth associated with cIII overproduction indicates that a prolonged maximal heat shock response might be highly deleterious; if so, the transient response would be an essential feature of the regulatory system. As one possible mechanism, transcription might be so directed to heat shock promoters that certain critical essential genes are underexpressed. The inhibition of growth might derive from some other activity of cIII besides its effect on σ^{32} ; however, the inhibitory capacity of cIII is strongly correlated with temperature, as is the activity of σ^{32} (Grossman et al. 1984). Moreover, extensive overproduction of σ^{32} from a plasmid in the absence of cIII provokes the heat shock response and is highly deleterious to bacterial growth at 30°C (Grossman et al. 1987; H. Bahl et al., unpubl.).

A second implication for the heat shock response is the indication that the regulatory system is controlled largely by the activity of σ^{32} . The cIII-mediated increase in the amount of σ^{32} at 30°C efficiently turns on the heat shock response. The normal heat shock response might involve an activation of the σ^{32} already present at 30°C, rapid synthesis of more σ^{32} , or stabilization of σ^{32} .

Implications for phage–host interactions during λ development

As noted in the introductory section, infection of *E. coli* by λ produces mildly elevated levels of at least some heat shock proteins (Draho and Hendrix 1982; Kochan and Murialdo 1982). On the basis of our plasmid results, we presume that cIII is at least the major inducer of these host proteins. Previous efforts to map the heat shock induction to λ genes have been equivocal; the data of Kochan and Murialdo (1982) are consistent with involvement of the cIII gene, whereas Draho and Hendrix (1982) suggest *ea10* or *ral*. For our plasmid system carrying *ea10*, *ral*, and cIII, the heat shock induction was completely dependent on active cIII. With the plasmid systems, the induction of the heat shock response is a

strong function of temperature (presumably σ^{32} activity) and rate of cIII production. Thus, the analysis with deletion mutants of phage λ might be complicated by numerous physiological aspects (e.g., how well the phage grow).

The possible implications of heat shock induction for phage–host interaction can be summarized as: Some heat shock helps, but too much does not permit normal bacterial or viral growth. Heat shock proteins are likely to be general contributors to survival from extreme stress (Krueger and Walker 1984; Neidhardt et al. 1984); thus, elevated production might help the host survive the viral infection, a helpful event for both host and phage in the lysogenic response favored by cIII. Certain heat shock proteins also participate directly in λ development (e.g., DnaJ, DnaK, and GrpE in λ replication and GroEL and GroES in morphogenesis) (Friedman et al. 1984; Ang et al. 1986; Bardwell et al. 1986). Efficient replication aids λ integration (Brooks 1965); however, the interaction with GroEL and GroES is not easily reconciled as a logical component of the lysogenic pathway. In this case, the phage might be taking advantage of the host protective response for its lytic development. Although there is not a simple unitary explanation, an induced heat shock response can in principle benefit both phage and host.

For the plasmid systems in which cIII production has been maximized, the prolonged induction of the heat shock response appears to be highly deleterious to the host. This situation may be somewhat analogous to infection of cells by *cro⁻cI⁻* mutant of λ , in which the absence of early-gene repression is lethal for phage and host (Eisen et al. 1975; Folkmanis et al. 1977; Georgiou et al. 1979). Although clearly a multigenic phenomenon, this “Tro” effect has a major component in the p_L operon of λ (Georgiou et al. 1979). Moreover, infection by *λcro⁻cI⁻* stabilizes σ^{32} produced from a plasmid (K. Tilly and C. Georgopoulos, unpubl.). Thus, overproduction of cIII is likely to contribute. We can infer that the expression of the cIII gene is normally translationally limited because production of cIII is increased by mutational changes in the cIII gene that do not change an amino acid but do reduce secondary structure at the initiation site (e.g., cIII_{s2} and cIII_{sp} in our plasmid constructions) (Altuvia and Oppenheim 1986; D. Knight, R. Crowl, and H. Echols, unpubl.). This translational limitation may serve to protect against too much cIII in a normal infection.

Although many of the interactions between the heat shock proteins and λ development remain to be sorted out, the capacity of cIII to stabilize σ^{32} and thus induce the heat shock response appears to be a clearly defined addition to the list of fascinating interactions involving λ and *E. coli*.

Materials and methods

Bacteria, plasmids, and media

The following *E. coli* strains were used as hosts for the plasmids described below: JM101F'*lacI^Q* (Messing 1983) was used with

plasmid pHBA1; MC1061F'*lacI*^Q (Casadaban and Cohen 1980) was used with plasmids pDS1 and pHBA1; and C600Su (McMacken et al. 1970) was used with pRK248cIts and pDK280 and derivatives. Two standard cIII-producing plasmids were used (see Fig. 1). Production of cIII from pDK280 and its derivatives was provided by thermal induction of λ *p_L* under control of thermosensitive λ cI protein from a compatible plasmid, pRK248, which carried the *cIts2* and tetracycline-resistance genes (Bernard and Helinski 1979). Production of cIII from pHBA1 was provided by IPTG induction of the *tac* promoter (de Boer et al. 1983). Plasmid pHBA1 was constructed by replacing the small *EcoRI*–*SalI* fragment of pKK223-3 (Pharmacia) with the *EcoRI*–*SalI* fragment of a cIII-overproducing plasmid pRC23/cIII (R. Crowl, unpubl.). This cIII segment consists of the *HaeIII*–*SalI* fragment of λ , representing most of the cIII gene (bases 33,439–33,249; Sanger et al. 1982) and a synthetic oligonucleotide which has the sequence (*EcoRI*–*HaeIII*): AATTCATGCAATAC*GCT¹ATC*GCAGGT*TGG. The synthetic sequence introduces four silent changes in the base sequence of the open reading frame for cIII [*¹ is the same change as the cIII_{s2} mutation of Knoll (1979a), based on unpublished sequence data of D. Knight and H. Echols]. The changes introduced in the base sequence reduce the stability of an mRNA secondary structure which probably inhibits efficient translation (D. Knight, R. Crowl, and H. Echols, unpubl.); the wild-type amino acid sequence is unaltered. The resultant cIII gene is designated cIII_{sp} for "superproducer." Plasmid pDK280 was constructed by replacing the small *HindIII*–*SalI* fragment of pACYC184 (Chang and Cohen 1978) with the *HindIII*–*SalI* fragment of λ carrying the cIII gene (Knight and Echols 1983). Variants of pDK280 were also constructed in the same way carrying either the cIII_{am611} mutation (pDK281) or the cIII_{s2}-overproducing mutation (pcIII_{s2}) (Knoll 1979a,b). The plasmid pDK520 with cIII under control of the *lac* promoter has been described (Knight and Echols 1983). The σ^{32} -producing plasmid pDS1 was constructed by inserting into the *Bam*HI site of pACYC184 a *Bam*HI fragment carrying the *tac* promoter and the *ipoH* gene (*EcoRV* fragment of pFN97; Neidhardt et al. 1983).

Bacteria were grown in M9 salts (Miller 1972) supplemented with MgSO₄ × 7H₂O (0.25 gm/liter), FeSO₄ × 7H₂O (0.5 mg/liter), glucose (0.2% [wt/vol]), vitamin B1 (1 µg/ml), 18 amino acids (no methionine or cysteine) at 20 µg/ml each. Where needed, antibiotics were added as follows: tetracycline, 15 µg/ml; ampicillin, 100 µg/ml; chloramphenicol, 30 µg/ml.

Labeling experiments

Logarithmic-phase cultures (OD₆₀₀ of 0.2–0.3) were induced for cIII production either by a temperature shift from 30°C to 42°C or by addition of 0.5 mM IPTG. At time points before and after induction, 1-ml aliquots were removed to prewarmed vials, and [³⁵S]methionine was added for a final concentration of 150 µCi/ml. For measurement of protein synthesis, labeling was terminated after 30 or 60 sec by addition of unlabeled methionine (300 µg/ml) and rapid chilling to 0°C. For measurement of stability, cultures were grown as described above, and σ^{32} production was induced for 15 min by addition of IPTG. After pulse-labeling of 3-ml aliquots with [³⁵S]methionine (100 µCi/ml) for 1 min, unlabeled methionine was added to 300 µg/ml, and aliquots of 500 µl were transferred at intervals into prechilled tubes and kept at 0°C. Samples were then centrifuged and resuspended in one-third volume buffer containing: 10% (wt/vol) glycerol, 10 mM DTT, 2.4% (wt/vol) SDS, 60 mM Tris-HCl (pH 6.7), and 0.1% bromphenol blue (one-dimensional gel electrophoresis); or 9.5 M urea, 2% (wt/vol) NP-40, 5% (vol/vol) β -mercaptoethanol, 1.6% (vol/vol) ampholines (pH 5–7), 0.4%

(vol/vol) ampholines (pH 3.5–10) (two-dimensional gel electrophoresis). Samples for one-dimensional gel electrophoresis were heated to 100°C in a water bath for 3 min and then subjected to electrophoresis on a 9–19% gradient SDS-polyacrylamide gel (Laemmli 1970). Two-dimensional gel electrophoresis was carried out by the O'Farrell procedure (1975). Gels were dried on filter paper and autoradiographed with Kodak XAR-5 film. To determine relative protein synthesis rates or protein stability, films were scanned with a Joyce–Lobel densitometer. The areas of protein peaks were determined with a Numonic planimeter and normalized to values of control proteins, for which synthesis (or stability) was not affected by the experimental conditions.

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