Regulation of the promoters and transcripts of *rpoH*, the *Escherichia coli* heat shock regulatory gene

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In *Escherichia coli* the product of the *rpoH* (*htpR*) gene, σ^{32} , directs RNA polymerase to initiate transcription from heat shock promoters at all temperatures. Transcription of the heat shock genes is increased when cells are exposed to high temperatures because of increased transcription initiation by σ^{32} -RNA polymerase. As a step toward understanding the regulation of the heat shock response we have examined the transcription of the *rpoH* gene. Using S1 mapping, promoter cloning, and in vitro transcription, we have identified the promoters and the terminator for the *rpoH* transcription unit. The *rpoH* transcripts are monocistronic and originate from at least three promoters. None of the promoters is recognized by σ^{32} -RNA polymerase. Two are recognized by σ^{70} -RNA polymerase and are active at both low and high growth temperatures. We do not know what form of RNA polymerase recognizes the third promoter. Transcripts from this promoter are abundant only at high temperature and are present after shift to the lethal temperature of 50°C, even at times when there are no detectable transcripts from the other *rpoH* promoters. The amount of *rpoH* mRNA increases fivefold by 8 min after shift from 30 to 43.5°C but *rpoH* mRNA synthesis increases by less than twofold, indicating that there is post-transcriptional control of the level of *rpoH* mRNA and presumably of σ^{32} .

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When cells, tissues, or even whole organisms are exposed to elevated temperatures they respond by increasing the synthesis of the heat shock proteins. This response to high temperature, termed the heat shock response, is similar in organisms from every biological kingdom (Neidhardt et al. 1984; Lindquist 1986). If cells are shifted from a low temperature to a higher one within their growth range, the induction of the heat shock proteins is transient. If the cells are transferred to temperatures above their growth range, the heat shock proteins are expressed at high levels for as long as the cells synthesize protein (Neidhardt et al. 1984; Lindquist 1986).

The functions of the heat shock proteins are not well understood. It is clear that some of the heat shock proteins are required for survival at high temperature and that expression of the heat shock proteins increases the length of time cells survive at temperatures too high for growth (Neidhardt et al. 1984; Lindquist 1986). Stimuli other than heat can induce the synthesis of various heat shock proteins (Neidhardt et al. 1984; Lindquist 1986). These stimuli include starvation for nutrients, viral infection, and exposure to ethanol, oxidants, UV radiation, amino acid analogs, or DNA-damaging agents. It

seems that the heat shock proteins help protect cells from a variety of adverse conditions.

The heat shock response in *Escherichia coli* is induced rapidly. The synthesis of the 20 or so heat shock proteins is increased within 1 min after shift from 30°C to a higher growth temperature, such as 43°C (Neidhardt et al. 1984). Maximum rates of heat shock protein synthesis are reached within 5–10 min, and by about 30 min after the shift the synthesis of heat shock proteins has declined to a new rate characteristic of the higher temperature (Neidhardt et al. 1984). The increased synthesis of heat shock proteins after shift to higher temperatures is caused by increased transcription initiation from heat shock gene promoters (Yamamori and Yura 1982; Taylor et al. 1984; Cowing et al. 1985). At all temperatures most of the heat shock gene transcripts come from promoters that are recognized by the form of RNA polymerase ($E\sigma^{32}$) that contains the 32-kD σ -subunit, σ^{32} (Cowing et al. 1985; Y. Zhou, J. Erickson, T. Yura, and C. Gross, in preparation). σ^{32} is the product of the *rpoH* (*htpR*, *hin*) gene (Grossman et al. 1984; Landick et al. 1984; Yura et al. 1984). Mutations in *rpoH* that reduce the amount or the activity of σ^{32} eliminate the increased synthesis of heat shock proteins after shift to high tem-

Figure 1. S1 nuclease mapping of the 5' ends of the *rpoH* transcripts. The schematic outlines the strategy for mapping the 5' ends of the *rpoH* transcripts. The top portion of the schematic is a restriction map of the *rpoH* promoter region. Promoters P1, P3, and P4 are drawn as arrows to indicate the direction of transcription. The position of the 5' end of mRNA-2 is marked with an arrowhead. Beneath the map the 5'-end-labeled [32 P]DNA fragments (32 P indicated by *) used for mapping are shown along with the observed patterns of protection. (Solid lines) Protection; (dashed lines) S1 nuclease sensitivity. The letters A, B, and C refer to the autoradiograms A, B, and C in which those DNA fragments were used. (A) A 1040-bp *NcoI*-*Sall* fragment 5'-end-labeled at the *NcoI* site was hybridized (at 45°C) with 50 μ g tRNA (lane 1) or 30 μ g in vivo RNA isolated from strain SC122 20 min after a shift from 30 to 42°C (lane 2). The protected fragments are marked with numbered arrows. In this experiment mRNA-4 was resolved into two species which we call 4a and 4b. DNA size markers were 5'-end-labeled 32 P-*HpaII* fragments from pBR322 (lane 3). (B) An 880-bp *Clal*-*Sall* fragment 5'-end-labeled at the *Clal* site was hybridized at 30°C to RNA isolated from SC122 8 min after shift from 30 to 42°C to map the start sites of mRNA-2, mRNA-3, mRNA-4a, and mRNA-4b by comparison with a chemical cleavage ladder of the same fragment. The symbols used for the DNA sequence ladders are Y (C + T), C, R (G + A), and G. (C) A 760-bp *HindIII*-*Sall* fragment 5'-end-labeled at the *HindIII* site was used (hybridizations at 30°C) to map the start site of mRNA-1 by comparison with a chemical cleavage ladder of the same fragment. In vitro RNA was synthesized with Eo⁷⁰ (see legend to Fig. 4A) and in vivo RNA was isolated from SC122 8 min after shift from 30 to 42°C. DNA sequence ladders are labeled as in (B).

perature (Neidhardt and VanBogelen 1981; Yamamori and Yura 1982; Tobe et al. 1984; Grossman et al. 1985). The demonstration that heat shock genes have promoters recognized by Eo³² (Grossman et al. 1984;

Cowing et al. 1985) led to the idea that the heat shock response is controlled by factors that affect the amount or the activity of Eo³² (Grossman et al. 1984). Some evidence suggests that increases in the amount of σ^{32} may

account for the induction of heat shock mRNA synthesis (Grossman et al. 1984; 1987; Gross et al. 1987). Consistent with this idea, Tilly et al. (1986) have shown that *rpoH* mRNA levels increase with kinetics similar to heat shock gene mRNA levels.

Because of the central role of σ^{32} in the heat shock response, we examined the transcription of the *rpoH* gene during steady-state conditions and after shifts to higher temperatures. We found that *rpoH* is the only gene in its transcription unit. There are at least three *rpoH* promoters, two of which are recognized by the most abundant form of RNA polymerase, $E\sigma^{70}$. $E\sigma^{32}$ does not transcribe the *rpoH* gene in vitro, and mutations in *rpoH* do not decrease transcription of *rpoH* in vivo, showing that *rpoH* is not a heat shock gene. The level of *rpoH* mRNA increases after shift to high growth temperatures but the synthesis of *rpoH* mRNA does not increase proportionately. We suggest that this reflects either a direct control of the stability of the *rpoH* mRNAs or indirect stabilization, due to increased translation of the *rpoH* mRNAs at high temperature.

Results

The locations of the 5' ends of *rpoH* mRNA

S1 mapping was used to locate the 5' ends of *rpoH* mRNA. Total cellular RNA was isolated from strain SC122 20 min after shift from 30 to 42°C, and the 5' ends of the *rpoH* mRNA species were mapped as described in Materials and methods and in the legend to Figure 1. Protected fragments corresponding to four mRNA species were seen: mRNA-1 (5' end near -220), mRNA-2 (5' end near -130), mRNA-3 (5' end near -90), and mRNA-4 (5' end near -80) (Fig. 1A). On some gels mRNA-4 was resolved into two species, which we call mRNA-4a and mRNA-4b. When RNA was isolated from strains MC4100, MG1655, CAG11042, P90A5c, or their derivatives, similar results were seen, with the difference that mRNA-2 was present only in strain SC122 (data not shown). Transcripts larger than mRNA-1 could have been distinguished from full-length DNA-DNA hybrids since the region of the probe homologous to the bacterial DNA was 276 bp shorter than the full-length probe. Overexposure of autoradiograms sometimes revealed a band that could have arisen from protection by transcripts initiating more than 400 bases upstream of mRNA-1. If such upstream transcripts exist they account for less than 1% of the *rpoH* mRNA.

Better estimates of the locations of the 5' ends of the four *rpoH* mRNAs were obtained by comparing the migration of the S1-resistant fragments with the migration of the products of DNA sequencing reactions done on the same probes used for S1 mapping. The 5' ends of mRNA-2, mRNA-3, mRNA-4a, and mRNA-4b were determined to be at positions -132 or -133, -88, -82 or -83, and -78, by comparison with the sequence ladder of the probe labeled at the *Clal* site (Fig. 1B). The 5' end of mRNA-1 was determined to be at position -221 by comparison with the sequence ladder of the probe labeled at the *HindIII* site (Fig. 1C). The results of these

experiments are summarized in Figure 1 with the positions of the 5' ends of the mRNAs and the promoters (see the following sections) indicated.

The DNA sequence immediately upstream of the *rpoH* gene is shown in Figure 2. The positions of the 5' ends of the mRNAs are indicated with arrows. The start site of mRNA-1 is located within what is probably the transcription terminator of the *ftsYEX* operon (Gill et al. 1986), which is located immediately upstream of *rpoH*. There are sequences that resemble $E\sigma^{70}$ promoters upstream of the 5' ends of mRNA-1, mRNA-3, and mRNA-4 (underlined in Fig. 2), but there is no sequence that looks like an $E\sigma^{32}$ promoter (Cowing et al. 1985).

The *rpoH* transcription unit

Based on the DNA sequence 3' to *rpoH*, Landick et al. (1984) and Yura et al. (1984) proposed the existence of a ρ -independent terminator immediately following the *rpoH* gene. The 3' end of the *rpoH* mRNAs was found at the predicted site (Fig. 3, lane 3) by S1 mapping in vivo RNA using a probe 3'-end-labeled at the *BssHII* site in *rpoH* (Figs. 3 and 9, below). We never observed a larger species of *rpoH* mRNA, suggesting that the majority of transcripts terminated at this site in vivo. Transcripts made in vitro with $E\sigma^{70}$ end at the same site indicating that the sequence functions as a ρ -independent terminator (Fig. 3, lane 2).

The positions of the 5' and 3' ends of the *rpoH* mRNAs and the DNA sequence of the region indicate that *rpoH* is the only gene in its transcription unit. Little or no transcription from the *ftsYEX* operon proceeds into the *rpoH* gene and the *rpoH* transcripts stop before reaching the *livJ* gene, which is just downstream of *rpoH* (Landick et al. 1984).

The origins of the *rpoH* mRNA species

Several approaches were used to determine which of the *rpoH* mRNAs are initiated from unique promoters and what forms of RNA polymerase are responsible for initiating the transcripts. The evidence discussed in the following paragraphs indicates that mRNA-1, mRNA-3, and mRNA-4 arise from distinct promoters. We do not know if mRNA-2 results from initiation from a unique promoter, P2, or from RNA processing or degradation.

The promoter cloning plasmid pKO1 (McKenney et al. 1981) was used to confirm that the DNA sequences upstream of the 5' ends of the *rpoH* mRNAs contain promoters. DNA fragments from the region presumed to contain the *rpoH* promoters were inserted in front of the *galK* gene of pKO1, and the galactokinase activity produced from the resulting plasmids was measured. The 342-bp *EcoRV* fragment (see Fig. 9, below) which was thought to contain all the *rpoH* promoters, was inserted into pKO1 in both orientations, creating pJEK-1 and pJEK-9. Of the two plasmids, only pJEK-1 has promoter activity (Table 1), demonstrating that the promoters are oriented toward *rpoH*. The 342-bp *EcoRV* fragment was cut with *HindIII* and both the upstream 235-bp fragment and the downstream 111-bp fragment were ligated into pKO1, creating pJEK-11 and pJEK-5. Both pJEK-11 and

Figure 2. The DNA sequence of the *rpoH* promoter region. The noncoding strand is shown. The sequence contains the 10 terminal codons of the *ftsX* gene, the intracistronic region, and the first 7 codons of *rpoH*. The first base of the *rpoH* initiation codon is numbered 0. The protein products are indicated below the DNA sequence. The start sites for mRNAs 1, 2, 3, 4a, and 4b are marked with arrows. Possible $E\sigma^{70}$ promoter -35 and -10 sequences located upstream of the starts of mRNA-1, mRNA-3, and mRNA-4 are underlined. An inverted repeat that is probably the *ftsX* transcription terminator (Gill et al. 1986) is underlined with head-to-head arrows. Restriction enzyme cleavage sites used for cloning or S1 mapping are indicated. The sequence shown was determined by Landick et al. (1984) and Yura et al. (1984) to position -192. The sequence from -127 to -278 was determined by Gill et al. (1986) and confirmed by us.

pJEK-5 had promoter activity (Table 1). These results, combined with those of the S1 mapping experiments, demonstrate the existence of at least two *rpoH* promoters; at least one responsible for mRNA-1 and/or mRNA-2 and at least one responsible for mRNA-3 and/or mRNA-4.

The *rpoH* gene was transcribed in vitro with $E\sigma^{70}$ and the products were identified using S1 mapping. In the presence of pure $E\sigma^{70}$ mRNA-1 and mRNA-4 were synthesized (Figs. 4A and 1C), meaning that promoters for mRNA-1 (P1), and for mRNA-4 (P4), exist and are recognized by $E\sigma^{70}$. In some experiments (data not shown) mRNA-4a and mRNA-4b were resolved, indicating that they both can be synthesized by $E\sigma^{70}$, but we do not know if mRNA-4a and mRNA-4b arise from different promoters. Both the relative and absolute amounts of mRNA-1 and mRNA-4 could be varied by changing the NaCl concentration or by using linear or supercoiled DNA, but P1 was always the stronger promoter (data not shown).

mRNA-3 was not synthesized in vitro with either pure $E\sigma^{70}$ or $E\sigma^{32}$. To see if mRNA-3 originated from a specific promoter, we asked if it could be synthesized in vivo in the absence of any upstream promoters. We used

a plasmid, pTFN97, that carries the *rpoH* gene preceded by P4 and the presumed P3 but which is missing the upstream promoter(s) P1 and P2. To eliminate any transcripts from plasmid sequences that could be substrates for RNA processing, a strong transcription terminator was inserted just upstream of P3 (at the *Hind*III site) in pTFN97. Potential chromosomal precursors of mRNA-3 were eliminated by using a strain, PM107, that has an IS1 element located between the *rpoH* promoters and the structural gene (R. Calendar, pers. comm.). We isolated RNA from PM107 carrying pTFN97, S1 mapped the *rpoH* transcripts, and found that mRNA-3 was present (Fig. 4B). Since no plasmid or chromosomal transcript existed that could be cleaved to yield mRNA-3, there must be a mRNA-3-specific promoter, P3.

Eσ³² does not recognize the rpoH promoters

No specific *rpoH* transcripts were made when pure $E\sigma^{32}$ was used in in vitro transcription reactions (data not shown). To confirm that $E\sigma^{32}$ did not recognize the *rpoH* promoters in vivo we used a mutant [*CAG9301 rpoH120::kan*] that lacks σ^{32} (Gross et al. 1987; Y. Zhou, J. Erickson, T. Yura, and C. Gross, in prep.). RNA was isolated from the *rpoH120::kan* mutant, and from

Figure 3. Identification of the *rpoH* terminator. The schematic shows the strategy for mapping the *rpoH* terminator. The symbols are as in Fig. 1. The *rpoH* terminator is represented as a stem-and-loop structure. The probe, 3'-end-labeled at the BssHII site, was hybridized at 50°C to tRNA (lane 1), RNA made in vitro with Eσ⁷⁰ (lane 2), 20 μg in vivo RNA from SC122 (lanes 3 and 5). DNA size standards (lane 4). DNA size standards mixed with a sample identical to that in lane 3 (lane 5). The probable locations of the 3' ends are indicated by a box around the sequence (Landick et al. 1984; Yura et al. 1984) which also shows the predicted secondary structure for the terminator.

its *rpoH*⁺ parent, and S1 mapped. Transcripts initiating from promoters P1, P3, and P4 were observed in both strains (Fig. 5), showing that σ³² was not required for their synthesis. Combined with the inability of Eσ³² to synthesize *rpoH* mRNA in vitro, these in vivo results indicate that σ³² is not necessary for the synthesis of any *rpoH* mRNA in these strains.

The relative amounts of the rpoH mRNAs vary with temperature

The experiments of Tilly et al. (1986) showed that the amount of *rpoH* mRNA varies with the growth tempera-

ture, but they did not distinguish the individual mRNA species. We used S1 mapping to see if the amounts of the *rpoH* mRNAs changed at different temperatures. RNA made at 30°C was compared with RNA made after shift to 43.5°C, at which temperature the heat shock response is transient, or with RNA made after shift to the lethal temperature of 50°C, at which temperature heat shock proteins are expressed at high rates for as long as the cells make protein (Neidhardt et al. 1984).

The predominant *rpoH* mRNA species at 30°C were the σ⁷⁰-controlled transcripts mRNA-1 and mRNA-4, together accounting for about 90% of the total *rpoH*

Table 1. Galactokinase activity from *rpoH* promoter-galK fusion plasmids

Plasmid	Promoters	Galactokinase activity at 30°C ^a	Relative plasmid copy number at 30°C ^b
pKO1	—	5.8	1.0
pJEK-1	P1 "P2" P3 P4	220	0.9
pJEK-9	4P 3P "2P" 1P ^c	17	NT ^d
pJEK-5	P3 P4	145	0.83
pJEK-11	P1 "P2"	155	0.85

Plasmid pJEK-9 was carried in strain CAG1573. The other plasmids were in strain CAG1831.

^a Galactokinase activity equals nmoles of galactose phosphorylated per minute per milliliter of cells per OD₆₅₀ unit.

^b Relative β-lactamase activity.

^c pJEK-9 has the same insert as pJEK-1 but in the opposite orientation.

^d [NT] Not tested.

mRNA. After shift to 43.5°C, the amounts of all the mRNAs increased (Fig. 6A), with mRNA-2 and mRNA-3 increasing the most. The increase in the level of mRNA-3 was delayed relative to the increase of the other *rpoH* mRNAs. This delay implies that the level of mRNA-3 is controlled in a different way than the level of the other *rpoH* mRNAs.

The relative amounts of the *rpoH* mRNA species at 50°C were different than at either 43.5°C or 30°C (Fig. 6B). The σ^{70} -controlled promoters P1 and P4 appeared to shut off gradually after shift to 50°C but mRNA-3 persisted at a high level for at least 85 min (data not shown). Nearly identical results (data not shown) were obtained when RNA was isolated from a strain carrying a temperature-sensitive mutation, *rpoD800*, that causes σ^{70} to be degraded rapidly after shift to high temperature (Grossman et al. 1983). If the continued presence of

Figure 4. Identification of the *rpoH* promoters. The strategy for S1 mapping was as in Fig. 1A. (A) In vitro transcription of *rpoH* with $E\sigma^{70}$. Probe was hybridized to 20 μ g of in vivo RNA prepared from strain SC122 3 min after shift from 30 to 42°C or to RNA synthesized in vitro by $E\sigma^{70}$ from pKP11 in 0.18 M NaCl, and S1 mapped. (B) mRNA-3 is synthesized from promoter P3. The schematic diagrams the plasmid and chromosomal *rpoH* templates in strain PM107/pTFN97. The thick line represents sequences from pDP6442; the transcription terminator is drawn as a stem-and-loop. The IS1 insertion in the chromosome of PM107 is shown by the hatched bar. The IS1 insertion was accompanied by a rearrangement or deletion of the sequences upstream of *rpoH*. Probe was hybridized to the following RNAs and S1 mapped: In vivo RNA isolated 8 min after shift from 30 to 43.5°C from strains SC122, PM107/pTFN97, PM107/pDP6442, and tRNA.

Figure 5. S1 mapping of in vivo *rpoH* transcripts in the absence of σ^{32} . Strategy was as in Fig. 1A. Probe was hybridized with tRNA (lane 1), in vivo RNA from strains MG1655 *rpoH*⁺ (lane 2), and CAG9301 *rpoH120::kan* (lane 3) grown at 16°C, or SC122 (lane 4) 20 min after shift from 30 to 43.5°C. DNA size standards (lane 5).

mRNA-3 reflected increased transcription initiation from P3 at a time when transcription from the σ^{70} -controlled promoters was declining, then this suggests that a different form of RNA polymerase was responsible for transcription from P3.

At 43.5°C the amount of rpoH mRNA increases without a significant increase in rpoH mRNA synthesis

The levels of *rpoH* mRNA increase with kinetics similar to those of the heat shock gene mRNAs after wild-type cells are shifted to high temperature (Tilly et al. 1986). The amount of *rpoH* mRNA increases five- to eightfold after shift from 30 to 43.5°C as measured using Northern blots, or by S1 mapping of the 3' or 5' ends of the *rpoH* mRNAs (Tilly et al. 1986 and Fig. 6A). The increased *rpoH* mRNA concentration could result either from increased transcription initiation or decreased degradation of the mRNA. We used two approaches to determine whether there was increased transcription initiation from the *rpoH* promoters after shift to high temperature.

The first approach was to measure the activity of the *rpoH* promoters before and after shift to high temperature using fusions between the *rpoH* promoters and the *galK* or *lacZ* genes. Plasmid pJEK-1 (Table 1) contains

Figure 6. S1 mapping of the *rpoH* transcripts at 30°C and at times after shift to 43.5 or 50°C. Mapping strategy was as in Fig. 1A. (A) Shift from 30 to 43.5°C. Probe was hybridized with 30 µg of in vivo RNA isolated from strain SC122 at 30°C (lane 1) or 4 min (lane 2), 8 min (lane 3), 12 min (lane 4), and 20 min (lane 5) after shift to 43.5°C. DNA size markers (lane 6). (B) Shift from 30 to 50°C. The amount of in vivo RNA used in each lane was the amount of RNA obtained from 2.5 ml of culture, corrected for sample recovery. Probe was hybridized to in vivo RNA isolated from SC122 at 30°C (lane 1), and 15 min (lane 2), 30 min (lane 3), 45 min (lane 4), and 60 min (lane 5) after shift to 50°C. DNA size markers (lane 6).

the *rpoH* promoters located in front of the *galK* gene. Transcription initiating from the *rpoH* promoters was quantified by determining the rates of synthesis of galactokinase at 30°C and after shift to 43.5°C. Cells containing pJEK-1 were pulse-labeled with [³H]leucine and [³H]lysine and the proteins separated on two-dimensional gels. The spots corresponding to galactokinase and the heat shock protein GroEL were cut from the gels and counted (Fig. 7A). Transcription from the *rpoH* promoters increased slightly, to approximately twice the 30°C rate, by 20 min after shift to 43.5°C. In contrast, the synthesis of the heat shock protein GroEL increased more than 12-fold by 10 min after shift and then declined before galactokinase synthesis increased significantly.

To confirm that the results obtained with the multicopy plasmid pJEK-1 mimicked the response of the *rpoH* promoters in single-copy, we constructed an operon fusion between the *rpoH* promoters and the *lacZ* gene of phage λRS45, creating λJEZ-30. We made λJEZ-30 lysogens of strain CAG11042 and examined the synthesis of β-galactosidase from this single-copy fusion by pulse-labeling the cells with [³⁵S]methionine and immunoprecipitating β-galactosidase. The results (Fig. 7B) are in qualitative agreement with those obtained with the multicopy plasmid pJEK-1 (Fig. 7A). There is no significant increase in transcription initiation from the *rpoH* promoters after shift to high temperature.

The second approach was to pulse-label cells with [³H]uridine and measure the rate of *rpoH* mRNA synthesis. There was not enough *rpoH* mRNA synthesis in wild-type cells to measure accurately, so the amount of *rpoH* mRNA was increased approximately 14 times by introducing the multicopy *rpoH*⁺ plasmid pKP11. S1 mapping was used to show that the relative magnitude and the kinetics of the increase in the level of *rpoH* mRNA after heat shock was similar in derivatives of CAG11042 with pKP11 or with the control plasmid pBR322. The magnitude of the heat shock response was similar in the two strains but the highest level of heat shock gene (*groE* or *dnaK*) mRNA was reached somewhat earlier in the strain with pKP11 (data not shown).

Since the relative increase in *rpoH* mRNA level was normal in the derivative of CAG11042 carrying pKP11, the strain was used to quantitate the synthesis of *rpoH* mRNA and of heat shock gene (*dnaK*) mRNA before and after transfer to 43.5°C. RNA synthesis was measured by hybridizing ³H-pulse-labeled RNA to *rpoH* or to *dnaK* DNA immobilized on nitrocellulose filters and measuring the ³H counts hybridized. mRNA levels were quantitated by S1 mapping nonradioactive RNA samples from the same cultures that were used for pulse-labeling. The data are depicted in Figure 8. The amount of *rpoH* mRNA increased fivefold, as seen previously (Tilly et al. 1986 and Fig. 6A), with a distinct peak at 8 min after shift. In contrast, there was a gradual and much

Figure 7. *rpoH* promoter activity after shift from 30°C to 43.5°C. (A) Synthesis of galactokinase from the *rpoH* promoters on plasmid pJEK-1 in strain CAG1831. The relative rates of galactokinase (●) or GroEL (▲) synthesis are plotted versus time after temperature shift. Rates of synthesis were determined by pulse-labeling and two-dimensional gel analysis as described in Materials and methods. (B) Synthesis of β -galactosidase from a single-copy *rpoH* promoter-*lacZ* fusion. A λ JEZ-30 lysogen of CAG11042 was pulse-labeled with [35 S]methionine at 30°C (lanes 1 and 2) and at 3 min (lane 3), 6 min (lane 4), 9 min (lane 5), and 20 min (lane 6) after shift to 43.5°C. Samples of equal 35 S cpm were immunoprecipitated with crude anti- β -galactosidase antiserum and detected by autoradiography. β -Galactosidase is indicated by the arrow.

smaller increase in *rpoH* mRNA synthesis. Both the synthesis and the level of *dnaK* mRNA increased and decreased as expected of a heat shock gene mRNA.

The results of the RNA pulse-labeling experiments and the promoter fusion experiments are consistent. There is a small increase in the synthesis of *rpoH* mRNA after shift to 43.5°C, but it is not enough to account for the fivefold increase in the *rpoH* mRNA concentration.

Discussion

Structure of the *rpoH* transcription unit

The *rpoH* gene is located between the *livJ* gene (Landick et al. 1984) and the *ftsYEX* operon at 76 min on the *E. coli* chromosome (Gill et al. 1986). The structural features of the *rpoH* transcription unit are diagrammed in Figure 9. In most of the strains we examined there are three monocistronic *rpoH* mRNA species, mRNA-1, mRNA-3, and mRNA-4. Each of the mRNAs originates from a promoter located immediately upstream of the 5' end of the mRNA. The promoters P1 (for mRNA-1) and P4 (for mRNA-4) are recognized by $E\sigma^{70}$. We did not determine what form of RNA polymerase recognizes P3, the promoter for mRNA-3. In strain SC122 there is a fourth mRNA (mRNA-2), but we have not established whether mRNA-2 results from processing or a specific initiation. If any other *rpoH* mRNAs exist they must account for less than 1–2% of *rpoH* transcription under the conditions (rich media) we used. The *rpoH* tran-

scripts stop at a ρ -independent terminator located 20–40 bp downstream of the *rpoH* coding sequence. Transcripts initiated from P1 and from P3 and P4 and ending at the *rpoH* terminator should be ~1.1 and ~1.0 kb in length. The conclusion that the *rpoH* transcripts are monocistronic is consistent with the results obtained from Northern blots by Tilly et al. (1986) who estimated the lengths of the *rpoH* transcripts to be ~1.4 and ~1.2 kb. Although their size estimates are larger than those made here, the estimate of 1.4 kb is too small to include *rpoH* and either the upstream *ftsYEX* operon or the downstream *livJ* gene. The different size estimates are probably due to difficulty in estimating the length of the RNAs using Northern blots.

The close genetic linkage between *rpoH* and the *ftsYEX* genes, which are involved in cell division, is worth noting given the reports that *rpoH* (Tsuchido et al. 1986) and at least two of the heat shock genes, *dnaK* (Paek and Walker 1987) and *lon* (Adler and Haridgree 1964; Howard-Flanders et al. 1964), are involved in cell division. Despite the reported link between *rpoH* and cell division, there is no evidence that the *ftsYEX* operon and *rpoH* are cotranscribed. It is possible that expression of the *ftsYEX* operon influences the expression of *rpoH* since the 3' end of the *ftsX* gene and the major *rpoH* promoter (P1) overlap; however, evidence suggests that there is no *cis*-acting effect of *ftsX* transcription on transcription of *rpoH* under normal growth conditions or during heat shock. Transcription of *rpoH* has been separated from transcription of *ftsX* using the *rpoH*⁺ plasmid pKP11, which lacks the *ftsYEX* promoters and all but the carboxy-terminal third of *ftsX*. Both the relative

Figure 8. Synthesis and accumulation of *rpoH* and *dnaK* mRNA. The relative increases in synthesis (dotted lines) and level (solid lines) of *rpoH* mRNA (●, ○) and *dnaK* mRNA (■, □) are plotted against time after shift from 30°C to 43.5°C. RNA was isolated from a culture of CAG11042/pKP11 grown at 30°C and at times after shift to 43.5°C. The mRNA levels were measured by quantitative S1 mapping of the 3' end of *rpoH* mRNA (Tilly et al. 1986 and Fig. 3) and the 5' ends of *dnaK* mRNA (Cowing et al. 1985) as described in Materials and methods. The rates of mRNA synthesis were determined by pulse-labeling with [³H]uridine and hybridizing [³H]RNA to filter-bound *rpoH* or *dnaK* DNA as described in Materials and methods. *rpoH*- or *dnaK*-specific RNA was determined by subtracting the ³H cpm bound to nonspecific DNA filters from the ³H cpm bound to the *rpoH* or *dnaK* filters.

amounts of mRNA-1, mRNA-2, and mRNA-4 (mRNA-3 is underrepresented from all multicopy plasmids tested) and the response of the *rpoH* transcripts to temperature shifts appear identical whether they come from pKP11 or the chromosome (data not shown). This suggests that transcription of *ftsX* does not directly influence transcription of *rpoH*.

Eσ³² does not transcribe the *rpoH* gene

RNA polymerase containing σ^{32} does not recognize the *rpoH* promoters in vitro, nor does it in vivo, since the 5' ends of in vivo *rpoH* mRNA are the same whether or not functional σ^{32} is present. These results mean that σ^{32} is not a heat shock protein, because by definition (Neidhardt et al. 1984) heat shock genes are transcribed by $E\sigma^{32}$. The same conclusion was reached by Bloom et al.

(1986) who were unable to demonstrate synthesis of σ^{32} in an in vitro transcription/translation system containing $E\sigma^{32}$, however, their conclusion was weakened by the fact that the *rpoH* template they used, pFN97, did not contain all the *rpoH* promoters. Some evidence suggests that the level of *rpoH* mRNA is higher after shift to high temperature in *rpoH* mutants than in wild-type cells (unpublished data and Fig. 3 of Tilly et al. 1986). This may mean that there is some form of autoregulation of σ^{32} synthesis, but we favor the idea that the increased level of *rpoH* mRNA in *rpoH* mutants reflects the existence of a feedback mechanism whereby the synthesis of σ^{32} is increased in response to a deficiency in heat shock protein synthesis. Such a mechanism may involve translational derepression of the *rpoH* mRNAs, as discussed later.

Figure 9. Diagram of the *rpoH* transcription unit. (Top) The *rpoH* gene is shown between the *ftsX* gene (Gill et al. 1986) and the *livJ* gene (Landick et al. 1984). Each gene is transcribed left to right as drawn in the figure. Promoters are indicated by a capital letter P and terminators by a small letter t. Restriction enzyme cleavage sites used for cloning or S1 mapping are shown. Symbols are: (E) *EcoRV*; (H) *HindIII*; (C) *ClaI*; (N) *NcoI*; (B) *BssHII*; (Hc) *HincII*. (Bottom) Expanded view of the *ftsX-rpoH* intracistronic region. The start sites of the *rpoH* mRNAs are indicated as arrowheads. Below the arrowheads the promoter responsible for each transcript (if known) is indicated. The wavy lines represent the *rpoH* mRNA species. The presumed transcription terminator of the *ftsYEX* operon (Gill et al. 1986) overlaps the start site of mRNA-1.

The increase in rpoH mRNA concentration is achieved by post-translational control

Tilly et al. (1986) reported that the amount of *rpoH* mRNA increases after shift to high temperature and that this increase is different from the induction of the heat shock gene mRNAs because it occurs in *rpoH* mutants. The increased *rpoH* mRNA concentration could have resulted from increased synthesis or decreased degradation of *rpoH* mRNA. Two mechanisms could lead to increased synthesis of *rpoH* mRNA; increased initiation of *rpoH* transcripts, or an antitermination control that would allow completion of more *rpoH* mRNA after shift to high temperature. We used three techniques to show that the increase in *rpoH* mRNA levels cannot be explained by increased synthesis. (1) S1 mapping was used to show that the 5' and 3' ends of the *rpoH* mRNAs increase to the same extent; this rules out any type of antitermination control of *rpoH* mRNA synthesis. (2) Fusions of the *rpoH* promoters to either the *galK* or *lacZ* genes were used to demonstrate that the promoters are not heat inducible. (3) The synthesis of *rpoH* mRNA was measured by pulse-labeling with [³H]uridine to show that *rpoH* mRNA synthesis increases only slightly in the first few minutes after shift to high growth temperatures. It seems likely that changes in the stability of the mRNAs accounts for the increased level of *rpoH* mRNA observed immediately after shift to 43.5°C since *rpoH* mRNA synthesis is not significantly increased during heat shock. It is possible to imagine conditions whereby a small increase in the synthesis of *rpoH* mRNA could lead to a larger increase in the *rpoH* mRNA level. For example, if *rpoH* mRNA were degraded at or near the maximum rate at 30°C, a small increase in synthesis of *rpoH* mRNA could saturate the degradation system thus

decreasing the rate of degradation and greatly elevating mRNA levels. The fact that the amount of *rpoH* mRNA can be increased more than 10 times, by the introduction of a multicopy *rpoH*⁺ plasmid, without changing the relative increase in *rpoH* mRNA levels before and after temperature shift argues against such a model.

Implications for the regulation of the heat shock response

Recent experiments have demonstrated that the concentration of σ^{32} transiently increases after shift to high temperature (Gross et al. 1987; Lesley et al. 1987; D. Straus, W. Walter, and C. Gross, in prep.). Our results suggest that the increase in *rpoH* mRNA level (and presumably σ^{32} synthesis) occurs by a post-transcriptional mechanism. We propose that the induction of σ^{32} synthesis occurs by post-transcriptional derepression and that the shut-off of σ^{32} synthesis is the reverse: post-transcriptional repression. This proposal is consistent with the experiments of Grossman et al. (1987) that implicate post-transcriptional control in the shut-off of σ^{32} synthesis. Repression or derepression of σ^{32} synthesis could occur either through direct control of mRNA stability or by regulation of translation.

Specific control of mRNA stability in response to environmental conditions is unusual in enteric bacteria. The control of the stability of *Klebsiella pneumoniae nif* mRNA in response to oxygen, ammonia, or temperature (Collins et al. 1986) and the growth rate dependence of *E. coli ompA* mRNA half-life (Nilsson et al. 1984) are two examples. If the *rpoH* mRNA concentration is raised by increasing the stability of the mRNAs at high temperature, it could be the result of a specific factor that stabilizes the mRNAs, or it could result from increased

translation of the *rpoH* mRNAs leading to increased stability, as in the case of r-protein mRNAs (Singer and Nomura 1985; Cole and Nomura 1986). We favor the second possibility, that increased σ^{32} synthesis leads to higher levels of *rpoH* mRNA, because the synthesis of σ^{32} peaks before the highest levels of *rpoH* mRNA are reached (Gross et al. 1987; D. Straus, W. Walter, and C. Gross, in prep.).

Is rpoH P3 recognized by a new form of RNA polymerase?

Of the four *rpoH* transcripts mRNA-3 stands out because of its unique response to temperature. At 30°C mRNA-3 accounts for less than 3% of the *rpoH* transcripts. After shift to 42 or 43.5°C the amount of mRNA-3 increases more than 10-fold (eventually amounting to about 15% of the total *rpoH* mRNA), although the increase is delayed relative to that of the other *rpoH* mRNAs and the heat shock gene mRNAs. The delay may mean that activation of P3 requires a factor produced in response to high temperature different from the factor that induces the heat shock response. At 50°C mRNA-3 accounts for nearly all of the *rpoH* mRNA.

It is possible that P3 is recognized by an undescribed form of RNA polymerase. Neither $E\sigma^{70}$ nor $E\sigma^{32}$ transcribe from P3 in vitro; however, preliminary experiments using extracts prepared from cells shifted to 50°C for 30 min suggest that mRNA-3 can be synthesized in vitro (data not shown). Because transcripts were not initiated from the σ^{70} -controlled promoters P1 and P4 in these extracts, we suspect that a form of RNA polymerase other than $E\sigma^{70}$ is involved in the synthesis of mRNA-3. The finding that mRNA-3 accumulates in vivo under conditions where the amount of σ^{70} should have been very low (the *rpoD800* mutant at 50°C) supports this idea. It is unlikely that $E\sigma^{32}$ is the other form of RNA polymerase since mRNA-3 is made in cells lacking σ^{32} (Fig. 5).

The exclusive presence of mRNA-3 at 50°C may reflect the existence of an emergency system to prolong survival at very high temperature. It is known that induction of the heat shock proteins provides protection from the lethal effects of high temperatures (Yamamori and Yura 1982). If the ability to synthesize heat shock

proteins is necessary for survival, it can be inferred that continual synthesis of σ^{32} would also be required since σ^{32} is unstable at 50°C (A. Grossman and W. Walter, unpubl.). Since transcription from the σ^{70} -controlled promoters P1 and P4 appears to be shut off at 50°C (Fig. 6B), *E. coli* may have evolved a mechanism to ensure continued transcription of *rpoH* from a heat-resistant promoter, P3, recognized by another form of RNA polymerase. This would allow continued expression of σ^{32} and might increase the chance of a cell surviving until the temperature was decreased. An attractive feature of this idea is that it provides a testable explanation of the nearly exclusive synthesis of heat shock proteins at 50°C. If σ^{70} is inactivated at 50°C but other σ factors including σ^{32} are not, the only proteins made will be those whose genes are recognized by forms of RNA polymerase other than $E\sigma^{70}$. The identification of proteins synthesized at 50°C may lead to the identification of genes that are recognized by alternate forms of RNA polymerase.

Materials and methods

Bacteria, phage, and media

The bacterial strains are listed in Table 2. Phage λ cl60dnaK[Am]dnaJ⁺ was obtained from C. Georgopoulos (University of Utah). λ rpoH⁺ (Y. Zhou, J. Erickson, T. Yura, and C. Gross, in prep.) is a derivative of Charon 25 that contains the bacterial DNA from pKP11. λ RS45 (Silhavy and Beckwith 1985) was obtained from R. Simons (University of California, Los Angeles). λ cl857 Sam7 DNA was purchased from Promega-Biotech, (Madison, Wisconsin). M9 (Miller 1972) or MOPS (Neidhardt et al. 1974) media were supplemented with glucose (0.2% wt/vol), vitamins, and 20 amino acids, except in pulse-labeling experiments where the amino acid or nucleoside used for labeling was omitted. When Amp^r plasmids were present, ampicillin (100 μ g/ml) was included in the culture medium.

Reagents

The Klenow fragment of DNA polymerase I, T4 DNA ligase, and T4 polynucleotide kinase were purchased from New England BioLabs (Beverly, Massachusetts). Restriction endonucleases were from New England BioLabs or Promega-Biotech. Calf intestine alkaline phosphatase, S1 nuclease, RNase A, and RNase T1 were from Boehringer-Mannheim Biochemicals (Indianapolis, Indiana). Antiserum against β -galactosidase was

Table 2. Bacterial strains

Designation	Genotype	Source
SC122	<i>lacZ</i> (Am) <i>trp</i> (Am) <i>pho</i> (Am) <i>mal</i> (Am) <i>supC</i> (Ts) <i>rpsL</i> <i>rel</i>	Cooper and Ruettinger (1975)
CAG1831	SC122 <i>galK2</i> <i>nadA50</i> : : Tn10	lab collection
CAG1573	<i>thi-1</i> <i>thr-1</i> <i>leu-6</i> <i>lacY1</i> <i>tonA21</i> <i>supE44</i> <i>galK2</i> <i>recA56</i>	lab collection
PM107	<i>rpoH107p</i> : : IS1 <i>rpoD285</i> <i>lacZ4</i> <i>argG75</i> <i>thi</i>	Grossman et al. (1985)
MG1655	prototrophic <i>E. coli</i> K12	CGSC ^a
CAG9301	MG1655 <i>rpoH120</i> : : <i>kan</i> <i>zhg21</i> : : Tn10	lab collection
CAG11042	F' <i>lacI</i> ^Q <i>lacZ</i> : : Tn5 <i>proA</i> ⁺ <i>B</i> ⁺ /Δ(<i>gpt-lac</i>)5 <i>trp</i> (Am) <i>thi</i> <i>ara</i> <i>galK2</i> <i>supC</i> (Ts) <i>rpsL</i>	from CSH 26, Miller (1972)
MC1061	Δ <i>lacX74</i> <i>galK</i> <i>galU</i> Δ(<i>ara-leu</i>)7697 <i>araD139</i> <i>hsdR</i> <i>rpsL</i>	M. Casadaban
MC4100	Δ(<i>argF-lac</i>)205 <i>araD139</i> <i>rpsL150</i> <i>thiA1</i> <i>relA1</i> <i>flb5301</i> <i>deoC1</i> <i>ptsF25</i> <i>rbsR</i>	M. Casadaban

^a CGSC *E. coli* Genetic Stock Center, Yale University.

provided by Boris Rotman (Brown University). Nitrocellulose filters were from Schleicher & Schuell, Inc. (Keene, New Hampshire) and yeast tRNA was from Sigma Chemical Co. (St. Louis, Missouri). Nitrocefin was from BBL Microbiology Systems (Cockeysville, Maryland). L-[³⁵S]Methionine (>800 Ci/mmol), L-[4,5-³H]leucine (52 Ci/mmol), L[4,5-³H]lysine (46 Ci/mmol), [5,6-³H]uridine (50 Ci/mmol), [γ -³²P]ATP (3000 Ci/mmol), [α -³²P]dCTP (3000 Ci/mmol), and [α -³²P]dGTP (3000 Ci/mmol) were from Amersham Corp. (Arlington Heights, Illinois). D-[1-¹⁴C]Galactose (40–50 mCi/mmol) was purchased from Research Products International Corp. (Mount Prospect, Illinois).

Plasmids

The Amp^r, *rpoH*⁺ plasmids pFN97 and pKP11 have been described (Neidhardt et al. 1983; Paek and Walker 1986). pKP11 contains 620 bp of *E. coli* DNA upstream of *rpoH*, the *rpoH*, *livJ*, and *livL* genes, and part of the *livK* gene inserted into *Bam*HI site of pBR322 (Bolivar et al. 1977). Standard techniques (Maniatis et al. 1982) were used for restriction enzyme digestions, small-scale isolation of plasmid DNA, agarose gel electrophoresis, DNA ligations, and transformations. When necessary, overhanging 5' ends were filled in with dNTPs using the Klenow fragment of DNA polymerase I. The structures of all plasmids were checked by restriction mapping. pTFN97 was created by inserting a blunt-ended *Hind*III to *Hinc*II fragment containing the *rpoH* gene from pFN97 into the *Eco*RI site of pDP6442 (obtained from D. Polayes and J. Dahlberg, University of Wisconsin). pDP6442 contains the transcription terminator from the *spf* gene upstream of the *Eco*RI site. The *spf* terminator has been shown to function with greater than 99% efficiency in vitro (Joyce and Grindley 1982). Two promoter cloning vectors were used, pKO1 (McKenney et al. 1981), carrying the *galK* gene encoding galactokinase, and pRS415 (Silhavy and Beckwith 1985, obtained from R. Simons), carrying the *lacZ* gene encoding β -galactosidase. All *rpoH* promoter fragments originated from pKP11. The fusions between the *rpoH* promoters and the *galK* gene of pKO1 are described in Results and in Table 1. The DNA fragments were made blunt-ended and inserted into the *Sma*I site of pKO1. pJEZ-30, a fusion of the *rpoH* promoters and the *lacZ* gene, was made by inserting a blunt-ended 880-bp *Sall*–*Cla*I DNA fragment containing the *rpoH* promoters into the *Sma*I site of pRS415.

Construction of a single-copy fusion of the *rpoH* promoters and the *lacZ* gene

The phage λ RS45 was constructed to allow transfer of gene fusions from plasmids to the phage by homologous recombination (Silhavy and Beckwith 1985). Strain MC1061 carrying pJEZ-30 was infected with λ RS45 and a recombinant Lac⁺ phage carrying the *rpoH* promoter–*lacZ* fusion was isolated and named λ JEZ-30. λ JEZ-30 lysogens of strain CAG11042 were purified from the centers of plaques and the number of prophage/cell was determined by the relative amounts of β -galactosidase produced.

Galactokinase and β -lactamase assays

Cells were lysed as described by McKenney et al. (1981), except that sodium deoxycholate (0.3% wt/vol) was included in the lysis buffer. Galactokinase activity was determined by measuring the conversion of galactose to galactose-1-phosphate (McKenney et al. 1981). β -Lactamase activity was assayed by measuring the hydrolysis of nitrocefin (Tomizawa 1985).

Protein labeling and measurements of protein synthesis

Cells growing in M9 glucose medium were pulse-labeled by transferring 0.5-ml samples to prewarmed tubes containing either 10 μ Ci of [³⁵S]methionine or 50 μ Ci of [³H]leucine and 50 μ Ci of [³H]lysine. After a 1-min pulse, excess nonradioactive methionine, or leucine and lysine, was added. For quantitative experiments, pulse-labeled ³H-samples were mixed with aliquots of ³⁵S-labeled extracts from the same strain to correct for sample recovery, prepared for two-dimensional gel electrophoresis (O'Farrell 1975), and quantitated as described in Grossman et al. (1987). For immunoprecipitations, ³⁵S-labeled samples were chilled on ice, centrifuged, and resuspended in 2% (wt/vol) SDS, 0.05 M Tris hydrochloride (Tris-HCl) (pH 7.5) and boiled for 2 min. The samples were immunoprecipitated (McKnight and Fried 1981) using antiserum against β -galactosidase. The immunoprecipitates were examined by one-dimensional SDS-polyacrylamide gel electrophoresis (Laemmli 1970).

RNA preparation

A modification of the method of Jinks-Robertson et al. (1983) was used to prepare RNA from cells growing in MOPS glucose medium. Cell samples of 5 or 10 ml were transferred into an equal volume of hot (95–100°C) lysis medium [1% (wt/vol) SDS, 0.008 M EDTA, 0.1 M NaCl] and incubated at 95–100°C for 1.5 min. An equal volume of water-saturated phenol was added and samples were shaken for 5 min at 64°C. After centrifugation to separate the phases, the phenol extraction was repeated. An additional extraction was done at room temperature with an equal volume of phenol/chloroform/iso-amyl alcohol (25 : 24 : 1) buffered with Tris-HCl (pH 7.9). The samples were precipitated twice with ethanol and resuspended in 0.01 M Tris-HCl (pH 7.5), 0.001 M EDTA and stored at –80°C. The RNA concentration was calculated by assuming that a solution of 40 μ g RNA/ml has a UV absorbance of 1.0 at 260 nm. When [³H]RNA was made, cells (1.0 ml) were transferred to a prewarmed tube containing 50 μ Ci of [³H]uridine; 30 sec later 1.0 ml of hot (95–100°C) lysis medium containing 0.20 mg of yeast tRNA was added. The remainder of the procedure was as described, except that the RNA was resuspended in filter hybridization buffer (0.1 M Tris-HCl (pH 7.5), 0.75 M NaCl, 0.002 M EDTA, 0.5% (wt/vol) SDS, 50% (vol/vol) deionized formamide). ³H incorporation was measured by trichloroacetic acid precipitation.

In vitro transcription

E σ ⁷⁰ was reconstituted from pure core RNA polymerase and σ ⁷⁰ (Lowe et al. 1979). E σ ³² was purified using the initial steps of Grossman et al. (1984) and chromatography on single-stranded DNA agarose and Sephacryl S400 (Lowe et al. 1979). E σ ³² was separated from core RNA polymerase and residual E σ ⁷⁰ by three passages through a Bio-Rex 70 column (Grossman et al. 1984). Reaction conditions were those of Grossman et al. (1984) except that pKP11 was the DNA template, all four NTPs were present at 0.2 mM, and the NaCl concentration varied between 0.05 M and 0.2 M. RNA was purified from in vitro reactions by extraction with water-saturated phenol followed by ethanol precipitation, and stored in 0.01 M Tris-HCl (pH 7.5), 0.001 M EDTA at –80°C.

Purification of plasmid and phage DNA

Plasmids were amplified (Maniatis 1982) and purified using the polyethylene glycol precipitation method of Humphries et al. (1975). λ phage and λ DNAs were isolated as described by Dunn and Blattner (1987).

DNA end-labeling and isolation of labeled fragments

Plasmid DNAs were cut with a restriction enzyme, dephosphorylated using calf intestine alkaline phosphatase, and labeled at the 5' ends with [γ - 32 P]ATP using T4 polynucleotide kinase (Maxam and Gilbert 1980) or DNAs were labeled at 3' ends using the Klenow fragment of DNA polymerase I and the appropriate [α - 32 P]dNTP (Maniatis et al. 1982). The end-labeled DNAs were cut with a second restriction enzyme and electroeluted from polyacrylamide gels using an IBI Model UEA electroeluter (International Biotechnologies, Inc., New Haven, Connecticut) according to the directions of the manufacturer. All 5'-end-labeled *rpoH* probes originated from pKP11. The fragments were 5'-end-labeled at the sites indicated in the figures and recut with *SalI*. The 3'-end-labeled probes came from either pKP11 or pFN97. They were 3'-end-labeled at the *Bss*HII site and recut with *HincII*.

S1 mapping

RNA-DNA hybridization conditions were those of Berk and Sharp (1978). All samples contained excess DNA. RNA samples (adjusted to 50 μ g of RNA per sample with tRNA) were hybridized to 0.1–0.5 μ g of labeled plasmid or the molar equivalent of purified restriction fragment for 14–16 hr at 45°C. S1 nuclease (170 units per reaction) reaction conditions were those of Berk and Sharp (1978), except that reactions were for 1 hr at 37°C. S1-resistant DNA fragments were electrophoresed in 6% (wt/vol) polyacrylamide gels containing 50% (wt/vol) urea (Maxam and Gilbert 1980). The experiments were quantitated by measuring the radioactivity in DNA fragments cut from the gels.

DNA sequencing

The method of Maxam and Gilbert (1980) was used.

Filter hybridizations

λ cl857 Sam7, λ rpoH⁺, or λ I60dnaK[Am]dnaJ⁺ DNA was denatured and bound to nitrocellulose filters (8.2 cm diameter) (Cooper 1972). Seven-millimeter-diameter circles containing about 5 μ g of DNA were prepared from the large filters with a hand punch and baked at 80°C for 3 hr in a vacuum oven. [3 H]RNA was hybridized to filter-bound DNA for 14–16 hr at 38°C in 1.0 ml of filter hybridization buffer [0.1 M Tris-HCl (pH 7.5), 0.75 M NaCl, 0.002 M EDTA, 0.5% (wt/vol) SDS, 50% (vol/vol) deionized formamide] containing 0.2 mg of yeast tRNA (modified from Court et al. 1980). Hybridizations contained 850,000 cpm [3 H]RNA and one *rpoH*-specific, one *dnaK*-specific, and one nonspecific (λ) filter. Filters were washed with 500 ml of 2 $\times$ SSC [0.3 M NaCl, 0.03 M sodium citrate (pH 7.0)], treated with RNase A (0.5 mg) and RNase T1 (500 units) for 30 min in 500 ml of 2 $\times$ SSC, rinsed with 500 ml of 2 $\times$ SSC, washed twice with 95% ethanol, dried, and counted in 5 ml of scintillation fluid. All hybridizations were done with excess DNA.

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