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Roles and regulation of the heat shock σ factor σ^{32} in *Escherichia Coli*

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When cells of *E. coli* are shifted to high temperature, the synthesis of a set of heat shock proteins is rapidly and markedly induced (see Neidhardt and VanBogelen, 1987). The induction occurs coordinately and transiently, mainly at the level of transcription (Yamamori and Yura, 1980; 1982). The molecular basis for such induction has been a subject of intensive studies during past years, and the central role of σ^{32}, the product of the *rpoH* gene (= *htpR*, *hin*) in the heat shock response is now well established (see Gross et al., 1990).

σ^{32} *plays an essential role in cell growth at high temperature*

Initial studies on the temperature-sensitive nonsense mutant (Tsn-K165; Cooper and Ruettinger, 1975) deficient in heat shock induction led to the proposal that the gene (*rpoH*) encodes a positive regulatory protein (Yamamori and Yura, 1982; Neidhardt and VanBogelen, 1981). This proposal was substantiated by the isolation and analysis of additional *rpoH* mutants (Tobe et al., 1984), by cloning and sequencing of the gene (Landick et al., 1984; Yura et al., 1984), and by direct analysis of the gene product which turned out to be the first minor form of σ factor (σ^{32}) discovered in *E. coli* (Grossman et al., 1984). Thus RNA polymerase containing σ^{32} was shown specifically to recognize a set of promoters mostly responsible for transcription of the heat shock genes (Grossman et al., 1984; Cowing et al.,1985).

Since *E. coli* mutants carrying a nonsense or missense *rpoH* mutation can grow at low temperature but not at high temperature, the *rpoH* gene product (σ^{32}) must play an essential role in cell growth at least at high temperature. On the other hand, the fact that a number of nonsense *rpoH* mutants could be isolated in a strain carrying no known tRNA suppressors (Tobe et al., 1984) suggested that σ^{32} may be dispensable at low temperature (Yura et al., 1984). This turned out to be the case, because *rpoH* null mutants lacking σ^{32} due to an insertion or deletion have been isolated and shown to be able to grow at or below 20°C (Zhou et al., 1988).

GroE and DnaK represent key heat shock proteins

The *rpoH* deletion mutant provided not only the convincing evidence that σ^{32} is the only σ factor that can recognize and transcribe from the heat shock promoters in vivo, but a unique opportunity to assess the physiological roles of heat shock proteins in cell growth. In *E. coli*, about twenty heat shock proteins have been identified by two-dimensional gel electrophoresis (Neidhardt and VanBogelen, 1987), and genes for nearly half of them are known. Among those studied in detail, some proteins including GroEL, GroES, DnaK, DnaJ and GrpE are essential for growth at all temperatures (see Georgopoulos, 1990),whereas others (Lon, LysU and HtpG) are dispensable under normal growth conditions.

Since most if not all of the heat shock proteins are drastically reduced in the *rpoH* deletion strain, revertants that can grow at higher temperature (30°C, 37°C or 40°C) were systematically analyzed to obtain new insight into the function of heat shock proteins. Interestingly,most revertants were found to produce increased but varying amounts of GroE due to an insertion upstream of the *groE* operon (Kusukawa and Yura, 1988). Moreover, the greater the expression of *groE*, the higher the permissive growth temperature, suggesting that, among the heat shock proteins, GroEL and GroES are uniquely required in large quantities for cell growth up to 40°C. In agreement with this, the *rpoH* deletion strain carrying a multicopy plasmid with the *groE* operon could grow up to 40°C.

To achieve growth above 40°C, an additional

mutation was required, and the second-step revertants turned out to overproduce another heat shock protein, DnaK. The requirement for DnaK was also confirmed by showing that the *rpoH* deletion strain carrying plasmids with the *groE* operon and *dnaK* could grow at 42°C (Kusukawa and Yura, 1988). These results suggest that among the heat shock proteins, only GroEL and GroES proteins are required in large amounts for growth up to 40 °C. For growth above 40°C, large amounts of DnaK are also required. So,GroEL, GroES and DnaK seem to represent key heat shock proteins required in large amounts for cell growth in the normal temperature range. However, this does not exclude a role for small amounts of heat shock proteins other than GroEL and GroES in growth at temperatures below 40°C. In fact, DnaK and GroE are known to be essential at all temperatures, so that limited expression of these genes probably occur from σ^{70} promoters. In fact,GroE itself is transcribed from a minor σ^{70} promoter, and 30% of normal level of GroE is produced in the *rpoH* deletion strain at 20°C (Zhou et al., 1988).

The role of σ^{32} and heat shock proteins in plasmid replication

It was recently found that expression of an F plasmid function essential for autonomous replication is directly controlled by σ^{32} (Wada et al., 1987). It was initially shown that the *rpoH* mutants are deficient in F plasmid maintenance, and segregate F^- cells at high frequency (Wada et al., 1986). Mini-F plasmids that essentially consist of an origin of replication (*ori2*) and the gene (*repE*) encoding a replication initiator protein exhibited similar instability. Promoter cloning and analysis of in vivo transcripts indicated that *repE* transcription depends on the *rpoH* function. The amount of *repE* mRNA increased transiently upon shift from 30°C to 42°C in the *rpoH*$^+$ (mini-F) strain, but decreased markedly at high temperature in the *rpoH* amber mutant carrying a temperature-sensitive suppressor tRNA. Indeed, the *repE* promoter sequence is similar to one of the heat shock promoters *PrpoD*$_{hs}$. Studies of transcription in vitro using purified RNA polymerase revealed that *repE* is transcribed primarily by σ^{32}-RNA polymerase, although minor RNAs are transcribed by σ^{70}-RNA polymerase (Wada et al., 1987). Furthermore, mini-F plasmid cannot transform *rpoH* deletion strains at any temperature unless the host bacteria contain a multicopy plasmid that can provide RepE function *in trans*. These results indicate that the supply of RepE protein is necessary and sufficient formini-F replication to occur in the host bacteria lacking σ^{32}.

Beside the direct role of σ^{32} in *repE* transcription, heat shock proteins DnaK, DnaJ and GrpE are required for replication of mini-F and mini-P1 plasmids (Ezaki et al., 1989; Bukau and Walker, 1989; Kawasaki et al., 1990; Tilly and Yarmolinsky, 1990). In the case of mini-F, the requirement for these heat shock proteins disappears when RepE protein is provided *intrans* from a multicopy plasmid, which explains why excess RepE protein suffices for mini-F replication in the absence of σ^{32}. These results suggest that the set of heat shock proteins are not absolutely required formini-F replication but they somehow facilitate functioning of RepE protein perhaps at the stage of DNA initiation complex formation, like in the case of λ phage DNA replication (see Georgopoulos et al., 1990). Thus replication of F and related plasmids is under σ^{32} control at two different levels: σ^{32} directly controls RepE synthesis,whereas indirectly controls RepE function by way of heat shock proteins.

The heat shock response is regulated by intracellular concentration of σ^{32}

Because of the rapid and marked increase in the expression of heat shock genes upon temperature up shift, it was initially suspected that an alteration of σ^{32} activity is involved. However, work inseveral laboratories using immunological techniques led to the finding that changes in the cellular level of σ^{32} are directly responsible for increased transcription from heat shock promoters (Straus et al., 1987; Lesley et al., 1987; Skelly et al., 1987). σ^{32} was found to be a vey unstable protein (half lefe = 1 min) and is rapidly degraded during steady-state growth (Straus et al., 1987; Tilly et al., 1989). However, σ^{32} is stabilized for several minutes after temperature upshift. In parallel with this transient stabilization of σ^{32}, the rate of σ^{32} synthesis is rapidly though transiently enhanced about tenfold. Thus both stabilization and increased synthesis of σ^{32} contribute to the rapid and transient increase in σ^{32} concentration observed during heat shock response.

Transcriptional control of rpoH

At least four promoters (P1, P3, P4 and P5) for *rpoH* transcription have been identified, and their relative activities vary with temperature and other conditions. Transcription from all promoters except P3 is mediated by σ^{70}-RNA polymerase, whereas transcription from P3 requires a new s factor (σ^E) which is markedly activated at high temperature (42-50°C) (Erickson and Gross, 1989; Wang and Kaguni, 1989b). The P5 promoter was recently shown to require cAMP and its receptor protein (CRP) for its activity, and sensitive to glucose inhibition (Nagai et al., 1990). Besides, one or more promoters appear to be modulated by DnaA protein which plays an important role in chromosomal DNA replication (Wang and Kaguni, 1989a; our unpublished result). In spite of these apparently elaborate regulation, *rpoH* transcription does not seem to be the primary site of regulation responsible for increased σ^{32} synthesis following temperature upshift. Thus transcriptional control of *rpoH* appears to be involved in maintenance of the proper basal level of expression at a variety of conditions,and becomes particularly important under special circumstances such as exposure to a lethal temperature.

Translational control of rpoH

The involvement of translational control in temperature-induced σ^{32} synthesis was first suggested by the experiments in which the expression of operon fusion and gene fusion between *rpoH* and the reporter gene *lacZ* was compared after temperature shift (Straus et al., 1987; Nagai et al., unpublished). When both the transcriptional and translational signals of *rpoH* are fused to *lacZ* (gene fusion), the rate of β-gal synthesis increase after temperature upshift just like the increased synthesis of authentic σ^{32}. When only transcriptional signals of *rpoH* are fused to *lacZ* (operon fusion), little increase in β-gal synthesis is observed. The translational signals are important for heat induction of fusion protein to occur. Furthermore, the promoter is not important in this case, because heat induction occurs even with *ara* promoter, so long as translational signals come from *rpoH* (Nagai et al., unpublished). A mutation (*suhB*) that enhances σ^{32} synthesis perhaps at the translational level has recently been isolated, though the precise mechanisms involved remain unknown (Yano et al., 1990).

To further localize the DNA/RNA region involved in the translational control, we have recently constructed a series of deletion (in-frame fusions), by deleting the C-terminal portions of coding sequence. Using the set of these fusions,the rate of synthesis of fusion protein at 30°C and after shift to 42°C was measured by pulse-labelling and immuno precipitation with anti-β gal serum. The results obtained are summarized as follows:

1. as deletion progresses toward N-terminal, expression at 30°C goes up (ca. 15 fold) beyond certain point, and heat inducibility disappears concomitantly,
2. when deletion extends to or beyond 19 b from the initiation codon, expression at both 30°C and 42°C becomes low.

We currently interpret these results by assuming that two separate regions of mRNA are involved in the post transcriptional control of *rpoH*: one region involved in repression and heat induction, and the other region required for potentially high level expression without respect to temperature (Nagai et al., unpublished).

Experiments are currently in progress to elucidate how σ^{32} synthesis is controlled at the post transcriptional level, and how heat shock signal is transmitted to the cellular apparatus controlling synthesis and stability of σ^{32}. At least three heat shock proteins (DnaK, DnaJ, GrpE) have been shown to negatively regulate both the synthesis and stability of σ^{32} (see Gross et al., 1990). How we could fit the above results with such a regulatory circuit would also be an important problem for future studies trying to understand the regulation of the heat shock response in *E. coli.*

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