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Competition between sigma factors for core RNA polymerase

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ABSTRACT

The switch of RNA polymerase specificity from early to late promoters of bacteriophage T4 is achieved by substitution of host sigma factor, σ^{70}, with the T4 induced factor, σ^{gp55}. However, overproduction of σ^{gp55} from an expression vector is not detrimental to Escherichia coli growth. Direct competition binding assays demonstrate that σ^{70} readily displaces σ^{gp55} from RNA polymerase and thereby reverses the promoter specificity of the enzyme. The displacement also occurs with the core enzyme modified by bacteriophage T4 infection. We postulate that an antagonist of σ^{70} should be formed in T4-infected cells to aid σ^{gp55} in the early/late switch.

INTRODUCTION

Coordinate gene expression in bacterial or bacteriophage development and during response to environmental stress is achieved through cascades of sigma factors which are interchangable subunits of RNA polymerase that confer upon the core enzyme specificities to different classes of promoters (1, 2). The central element of such regulation is the act of substitution of one sigma factor by another. The biochemical parameters that govern this substitution, such as relative affinities of the sigma factors to the core enzyme, thus become the key to the understanding of how sigma cascades work.

In this work, we compared the interaction with the core enzyme of two sigma factors, σ^{70}, the principal initiation factor of E. coli, and σ^{gp55}, the central component of the late gene transcription apparatus of bacteriophage T4. This protein, encoded by T4 gene 55 has a molecular weight of 22,000 and is sufficient to direct E. coli core enzyme to utilize T4 late promoters in vitro (3, 4). During T4 development, the emergence of σ^{gp55} in the cell coincides with a transcriptional switch from early to late genes, which involves both activation of late promoters and turning off of early promoters which are normally recognized by host σ^{70} (5). Obviously, the substitution of σ^{70} by σ^{gp55} is responsible for this specificity switch.

What is the mechanism of the substitution? The simplest model would be to imagine that newly synthesized σ^{gp55} outcompetes σ^{70} in the infected cell by direct mass action. To address this question we analysed the competition between the two factors in vivo and in vitro.

MATERIALS AND METHODS

Bacterial Strains. σ^{70} overproducing strain, M5219(pMRG8) (6) was obtained from M. Gribskov. σ^{gp55} overproducing strain (7) was obtained from W. Rüger.

Enzyme Preparations. Core enzymes from uninfected and T4-infected E. coli were isolated as described previously (8). σ^{70} was purified from the overproducing cells as described (6). Purification and properties of σ^{gp55} and the characterization of the enzyme and factor preparations used in this work are presented elsewhere (9).

For the preparation of radioactive factors, the overproducing strains were grown in 0.5 l of minimal M9 medium supplemented with 50 µg/ml l-tryptophan, 50 µg/ml biotin, 0.4% glucose and 50 µg/ml ampicillin, and heated to 42°C. After 5 min, 100 µCi of ^{14}C-amino acid mixture (Amersham) was added and the cells were harvested one hour later. The radioactive factors were isolated using the same protocol as the unlabelled factors (6, 9). The specific radioactivity of the factors was about 1,800 cpm/µg (39,600 cpm/nmol) for σ^{gp55} and 7,500 cpm/µg (525,000 cpm/nmol) for σ^{70}.

Sigma Competition Binding Experiments. 0.28 nmoles of core enzyme was incubated with the indicated amount of the first sigma factor in 150 µl of TGED (6) containing 0.2 M NaCl for 10 min at 37°C. The indicated amount of the second sigma factor was added and the incubation was continued for another 10 min. 30 µl of glycerol was added, the sample was cooled in an ice water bath and applied to a 20 ml (0.7 x 50 cm) column of Sephacryl S-200 (Pharmacia) equilibrated with TGED + 0.05 M NaCl containing 0.1 mg/ml bovine serum albumin. The chromatography at the flow rate of 10-12 ml/h was performed at 4°C. 0.33 ml fractions were collected, mixed with Aquasol (New England Nuclear) LSC Cocktail and the radioactivity was counted. The identity of the proteins in the radioactivity peaks was confirmed by analysing the fractions by SDS gel electrophoresis.

Runoff Transcription Assay. The transcription system contained 40 mM Tris-HCl, pH 7.9; 10 mM $MgCl_2$; 50 mM KCl and 7 mM 2-mercaptoethanol. In a 100 µl reaction, RNA polymerase (5 µg) was preincubated with P7-8 template DNA fragment (50 ng) in the presence of 0.25 mM of the priming dinucleotide, UpG.

After 15 min, poly dA-dT at 100 μg/ml was added and preincubation continued for another 3 min. Transcription was initiatied by adding the four nucleoside triphosphates to a concentration of 5 μM each, maintaining the specific radioactivity of [^{32}P]UTP at about 2 Ci/mmole. After 15 min at 37°C, nucleotide concentration was raised to 0.4 mM each and the incubation continued for another 10 min. The reactions were stopped, the samples processed and analyzed in 10% polyacrylamide slab gels containing 8 M urea as described previously (10).

RESULTS

Competition Between the Two Initiation Factors in vivo

The scheme of sigma displacement based on simple mass action predicts that overproduction of σ^{gp55} in E. coli would be detrimental to the cell because the excess of the viral factor would titrate the core enzyme and make it unavailable for the utilization of host promoters by σ^{70}. Contrary to this prediction, we found that the overproducing strain carrying pLHg55 forms colonies and lawns at 42°C when plasmid-borne gene 55 is fully induced. In this construction, gene 55 is under the control of λ P_L promoter and heat labile repressor cI857. Upon heat induction, a substantial accumulation of σ^{gp55} takes place reaching about 20% of the total cellular protein after one hour at 42°C. At least some fraction of this protein is biologically active since the overproducing strain supports the development of a T4 mutant in gene 55 both at 30°C and 42°C (data not shown). Thus, σ^{gp55} fails to compete with σ^{70} in uninfected bacterial cells.

σ^{gp55} is displaced by σ^{70} from the holoenzyme

In the next series of experiments we analysed the interaction of the two factors with core RNA polymerase in direct binding assays. For this purpose we used radioactively labelled and unlabelled preparations of σ^{gp55} and σ^{70}. Free factors can be easily separated from their respective holoenzymes on a gel filtration column. In Figure 1, Panels A, B and C present the results for radioactively labelled σ^{70}, and Panels D, E and F for radioactively labelled σ^{gp55}.

It is evident that in the presence of an excess of core enzyme, radioactive σ^{70} is recovered in the high molecular weight fractions (open symbols, Panel A) away from the position of free σ^{70} (filled symbols). The stoichiometry of this effect is close to 1 as is evident from a shoulder which appears at equimolar ratio of sigma to core enzyme (broken line, Panel A). This shoulder becomes more prominent at higher sigma/core ratios (not shown). The addition of two-fold molar excess of unlabelled competitor σ^{70} results in a proporti-

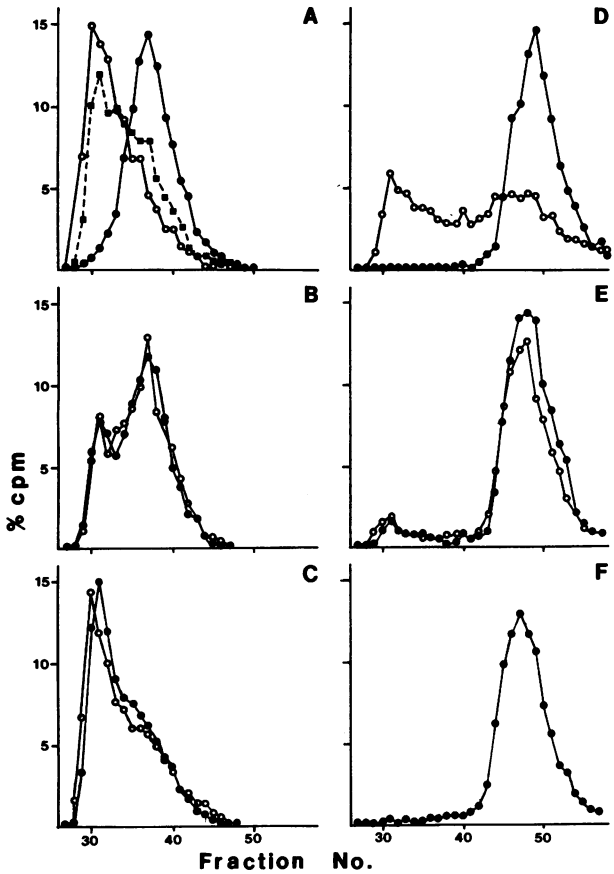

Fig. 1: Competition Binding of Sigma Factors to Core Enzyme.

^{14}C-σ^{70} (A, B and C) or ^{14}C-σ^{gp55} (D, E and F) was incubated with core and competitors under the conditions specified below, followed by fractionation on a Sephacryl column as described in Materials and Methods. The curves represent percent of total radioactivity recovered in the fractions.

Panel	Curve	Order of addition and molar ratios (in parentheses)
A	●—●	^{14}C-σ^{70}
	o—o	core(1.0) + ^{14}C-σ^{70}(0.05)
	■----■	core(1.0) + ^{14}C-σ^{70}(0.9)
B	●—●	core(1.0) + ^{14}C-σ^{70}(0.9) + cold σ^{70}(1.8)
	o—o	core(1.0) + cold σ^{70}(1.8) + ^{14}C-σ^{70}(0.9)
C	●—●	core(1.0) + ^{14}C-σ^{70}(0.9) + cold σ^{gp55}(9.0)
	o—o	core(1.0) + cold σ^{gp55}(90.0) + ^{14}C-σ^{70}(0.9)
D	●—●	^{14}C-σ^{gp55}
	o—o	core(1.0) + ^{14}C-σ^{gp55}(0.9)
E	●—●	core(1.0) + ^{14}C-σ^{gp55}(0.9) + cold σ^{gp55}(4.5)
	o—o	core(1.0) + cold σ^{gp55}(4.5) + ^{14}C-σ^{gp55}(0.9)
F	●—●	core(1.0) + ^{14}C-σ^{gp55}(0.9) + cold σ^{70}(4.5)

onal displacement of radioactive σ^{70} from the holoenzyme (Panel B). Note that this displacement does not depend on whether the unlabelled competitor is added before or after the radioactive factor (open and filled symbols). These results clearly demonstrate that holoenzyme, core and σ^{70} exist in equilibrium which is established faster than the 10 minutes incubation period prior to gel filtration. In spite of this fast equilibrium, sigma factor is not lost during gel filtration chromatography which lasts several hours. This apparent contradiction is resolved if we assume that the equilibration schedule between included and excluded material on the column is relatively long in comparison with sigma/core equlibrium. At high binding constant of sigma - core interaction, the dissociating factor will be effectively trapped in the core zone throughout chromatography.

This effect is apparently insufficient to completely retain σ^{gp55} within the core zone. As is evident from the results of Fig. 1, Panel D, the colum profile of σ^{gp55} preincubated with the core enzyme (open symbols) is smeared between the position of the holoenzyme and the position of the free factor (filled symbols). Under our conditions of gel filtration, by the end of the run, about 50% of the core molecules still contain σ^{gp55}. This proportion could not be increased by adding molar excess of radioactive σ^{gp55} (data not shown) which rules out the possibility that our σ^{gp55} preparation contains a subpopulation of molecules that fail to bind to core. The loss of σ^{gp55} during gel filtration indicates that its association with the core is much weaker than that of σ^{70}.

The addition of unlabelled competitor σ^{gp55} results in a proportional decrease in the amount of radioactive σ^{gp55} in the holoenzyme peak (Panel E). This effect is independent of the order of addition of the radioactive factor and the competitor (filled and open symbols), which is indicative of a true equilibrium between the holoenzyme, free core and σ^{gp55} in solution.

Thus, both σ^{70} and σ^{gp55} exchange quite rapidly between free and core associated forms. To find out whether the two factors compete with each other, heterologous unlabelled competitors were added to the binding reactions. The result (Panels C and F) revealed a dramatic difference between the host and T4 factors. σ^{70} readily displaced σ^{gp55} from the holoenzyme (Panel F) whereas a large excess of σ^{gp55} had no effect on the binding of σ^{70} (Panel C). In fact, radioactive σ^{70} could quantitatively bind to the core in the presence of 100 fold molar excess of competitor σ^{gp55} (Panel C, open symbols).

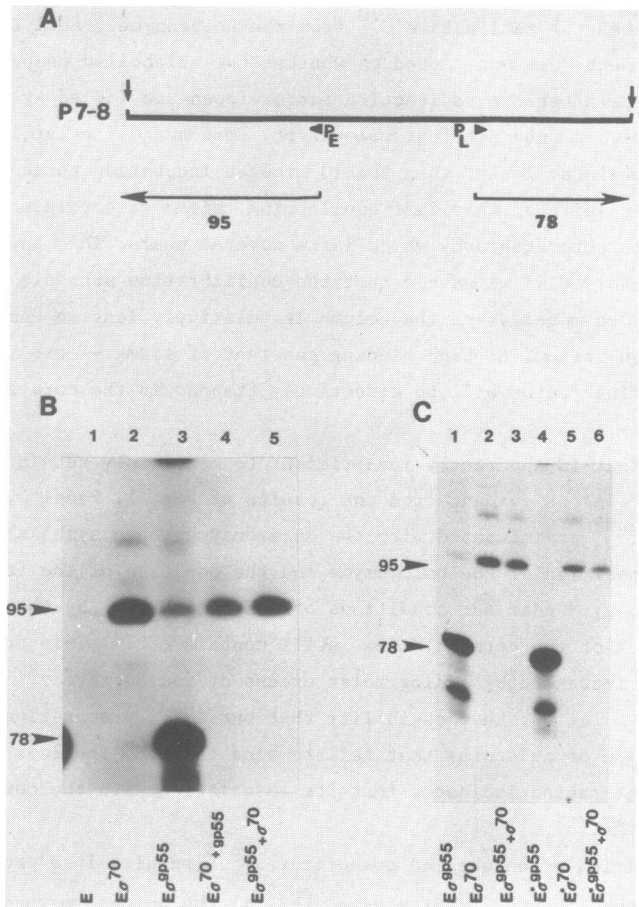

Fig. 2.: Change of Promoter Specificity by Sigma Displacement.
A. A scheme of the P7-8 template DNA fragment with two promoters and their runoff in vitro transcripts. The isolation and characterization of template properties of this 247 base-pair segment of T4 genome is described in Ref. 9. It carries an early (P_E) and a late (P_L) promoters which give rise to the transcripts represented by arrows.
B. Transcripts made on the P7-8 fragment by holoenzymes assembled from E. coli core (E) and indicated sigma factors. After assembly period (E incubated with the first sigma factor for 10 min at 37°C) the second sigma factor was added where indicated for another 10 min, followed by runoff transcription assay. The molar ratio of E to the first factor to the second factor was 1:1:5.
C. Same as B, using core enzymes from uninfected (E) or T4-infected (E*) E. coli.

Displacement of σ^{gp55} by σ^{70} Changes Promoter Specificity

The *in vitro* runoff transcription system using purified DNA fragment P7-8 as template (9) is employed in our laboratory for the functional assay of the two factors. As is shown in the scheme of Fig. 2A, the template fragment contains two major transcription start sites responding to the two sigma factors. The rightward σ^{gp55}-dependent promoter, P_L, gives rise to a 78 nucleotide long runoff transcript (Fig. 2B, Lane 3). The leftward promoter, P_E, gives rise to a runoff product about 95 nucleotides long (Fig. 2B, Lane 2). (Traces of this transcript found in $E\sigma^{gp55}$ product (Fig. 2B, Lane 3) are probably due to a slight contamination of our core enzyme with σ^{70}). Neither of the promoters is utilized by the core enzyme alone (Lane 1).

When an excess of σ^{gp55} was added to preformed $E\sigma^{70}$ holoenzyme before transcribing P7-8 template DNA (lane 4), the predominant product was the 95 nucleotide transcript initiated at P_E. Thus, σ^{gp55} cannot displace σ^{70} from holoenzyme. In the experiment of Lane 5, an excess of σ^{70} was added to preformed $E\sigma^{gp55}$ holoenzyme. The predominant product is still the 95 nucleotide P_E-driven transcript. This result confirms the conclusion of the previous section that σ^{70} outcompetes σ^{gp55} in the race for binding with the core enzyme.

Exchange of Sigma Factors on T4-Modified Core

The failure of σ^{gp55} to compete with the host factor raises the question of its mode of action *in vivo* in T4-infected bacteria. One possibility is that other T4-induced modifications of RNA polymerase, which are not present in our *in vitro* assay, increase the affinity of σ^{gp55} to the core enzyme. Accordingly, we repeated the factor competition experiment using the core enzyme isolated from T4-infected cells. This core enzyme contains two modifications whose function is not known: it carries the 15,000 dalton T4 *rpbA* gene product (12), and its α-subunits are ADP-ribosylated by T4-specific enzyme (8). As is evident from Fig. 2C, host sigma factor displaces σ^{gp55} from T4-modified holoenzyme as easily as from unmodified polymerase.

DISCUSSION

The principal finding of this research is that the major sigma factor of *E. coli*, σ^{70}, readily displaces the substitute factor, T4-specified σ^{gp55} from RNA polymerase holoenzyme in solution. The same conclusion was reached by Williams et al. (11) in similar types of experiments.

The demonstration that σ^{70} outcompetes σ^{gp55} explains why the overproduction of σ^{gp55} is not detrimental for *E. coli*. It is reasonable to assume that during T4 development, the competitiveness of σ^{70} is somehow reduced in

order to give σ^{gp55} a chance to form the holoenzyme. One way to achieve this would be to provide an antagonist which would inactivate the host factor. Alternatively, a core enzyme modification could stabilize the $E\sigma^{gp55}$ holoenzyme so that σ^{gp55} would no longer be displaced by σ^{70}. Our results demonstrate that two of the many known RNA polymerase modifications (13), the 15K protein and the ADP-ribosylation of α-subunits are not sufficient for preventing the sigma displacement. This result, which is also reported by Williams et al. (11), is not surprising because both 15K protein (14) and ADP-ribosylation (15) are not essential for the early/late switch *in vivo*.

Another factor which could be involved in antagonizing σ^{70} is the product of T4 gene 33, a core binding protein which is essential *in vivo* for the early/late switch (16). Gene 33 has been recently placed on an expression vector (7) and we are currently trying to purify it in order to analyse its effect on sigma displacement. Another candidate for the role of σ^{70} antagonist is the 10,000 dalton T4-induced protein which has been shown to have an anti-σ^{70} activity *in vitro* (17).

Our results clearly demonstrate that σ^{70} and σ^{gp55} are indeed alternative factors in the sense that they cannot coexist in the same holoenzyme molecule in solution. One interpretation is that they compete for the same site on the core enzyme and that their equilibrium binding constants differ by at least two orders of magnitude so that σ^{70} can bind even in the presence of a large excess of σ^{gp55}. Alternatively, the displacement of σ^{gp55} by σ^{70} can be explained by an active mechanism which requires the formation of a transient two factor complex $E\sigma^{70}\sigma^{gp55}$:

$$\sigma^{70} + \sigma^{gp55} + E \rightleftharpoons \begin{matrix} E\sigma^{gp55} + \sigma^{70} \\ \searrow \\ [E\sigma^{70}\sigma^{gp55}] \\ \swarrow \\ E\sigma^{70} + \sigma^{gp55} \end{matrix}$$

In the latter case, the two factors should have separate or overlapping binding sites on the core molecule, so that the attachment of σ^{70} would cause the dissociation of σ^{gp55} either through an allosteric change or by simply increasing its own local concentration. Both schemes are consistent with the observation that homologous factors undergo fast exchange in solution, hence our present results do not allow us to distinguish between them.

Recently it was reported that σ^{70} can also displace from the holoenzyme

σ^{32}, the initiation factor of E. coli heat shock genes (18). This observation is consistent with the evidence that σ^{32} is present in non heat-shocked E. coli without causing transcription of heat shock genes (19, 20). Hence its placement upon core enzyme during heat shock response could be achieved not through simple mass action but with the help of an additional factor(s). On the other hand, the expression of σ^{22}, the sporulation factor from B. subtilis, appears to be lethal for E. coli cells (21). The authors suggest that successful competition with σ^{70} is responsible for this effect. All sigma factors share a homology region capable of folding into a beta sheet which was postulated to be the core binding site (22). It would be interesting to know whether the differences in the amino-acid sequence in the presumed core binding domains of these factors are responsible for their displacement relationships. We intend to use in vitro mutagenesis of T4 gene 55 to test this possibility.

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