Amino-terminal amino acids modulate σ-factor DNA-binding activity

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Prokaryotic transcription initiation factor σ is required for sequence-specific promoter recognition by RNA polymerase. Genetic studies have indicated that σ itself interacts with DNA at the -10 and -35 promoter consensus sequences. Binding of *Escherichia coli* σ70 to DNA in vitro, however, can only be observed for truncated polypeptides lacking the amino-terminal amino acids. We have investigated the role of the amino terminus of *E. coli* σ70 in controlling DNA-binding ability. Deletion analysis indicates that amino acids within amino-terminal region 1.1 of σ70 inhibit DNA binding by the carboxy-terminal DNA-binding domains. Furthermore, inhibition of binding by the amino-terminal inhibitory domain of σ70 can be observed in trans. Likewise, the amino-terminal extensions of two alternative σ-factors, *E. coli* σ32 and *Bacillus subtilis* σ8, negatively affect the DNA binding activity of their carboxy-terminal domains. We propose that initiation of transcription is subject to modulation as a result of the composition and/or structure of the amino terminus of the σ-subunit and that the σ family of proteins belong to a larger class of intramolecularly regulated transcriptional effectors.

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Initiation of transcription at prokaryotic promoter DNA sequences requires one of the σ-subunits of bacterial RNA polymerase [Burgess et al. 1969; Burgess and Travers 1970]. There are two distinct families of σ-factors. One family includes σ factors that are related to σ70, the *Escherichia coli* σ-factor required for nitrogen-regulated gene expression. The majority of known σ-factors, however, belong to the other family whose members are evolutionarily related to σ70, the *E. coli* primary σ-factor. The σ70 family is comprised of highly homologous proteins that have been subdivided into two groups: (1) the primary σ-factors, which are required for vegetative growth of the bacterial cell, and (2) the alternative σ-factors, which participate in the transcriptional response to changing environmental conditions or physiological states [Stragier et al. 1985; Gribskov and Burgess 1986; Helmann and Chamberlin 1988; Gross et al. 1992; Lonetto et al. 1992]. Both primary and alternative σ-factors from the σ70 family are subjects of this report.

The particular σ-factor associated with RNA polymerase holoenzyme provides specificity for promoter sequence recognition by the enzyme, leading to the proposal that σ-factors are sequence-specific DNA-binding proteins [Losick and Pero 1981]. Genetic studies have implicated two highly conserved regions of σ in promoter recognition. Mutations in region 2.4 of σ affect the ability of the holoenzyme to discriminate between base pairs in the –10 hexamer of the promoter consensus sequence, and mutations in conserved region 4.2 affect recognition of the –35 hexamer [Gardella et al. 1989; Zuber et al. 1989; Daniels et al. 1990; Waldburger et al. 1990]. Recently, a biochemical analysis of σ–promoter interactions showed that partial polypeptides carrying conserved region 2 or 4 of σ70 bind independently to promoter DNA with appropriate specificity, providing further support for the idea that σ itself can recognize and interact with specific DNA sequences [Dombroski et al. 1992].

Although the isolated DNA-binding domains of σ70 specifically recognize promoter DNA, full-length σ, in the absence of the core subunits of RNA polymerase (α2ββ'), does not interact specifically or nonspecifically with DNA [Burgess et al. 1969; Zillig et al. 1970; Wu et al. 1975; Kudo and Doi 1981; Kudo et al. 1981; Ramesh and Meares 1988; Wellman and Meares 1991]. At least for σ70, the amino-terminal region of σ appears to block the DNA-binding capacity of the more carboxy-terminal portion of the protein [Dombroski et al. 1992]. In this report we further characterize the amino-terminal inhibitory domain of σ70 and its mechanism of inhibition. Additionally, we have analyzed the DNA-binding properties of several alternative σ-factors. Our results indicate that the amino-terminal regions of both the primary and alternative σ-factors participate in modulation of DNA-binding activity, thus revealing a powerful mech-
anism for transcriptional regulation through manipulation of the \(\sigma\)-subunit of RNA polymerase. Implications for the mechanism of transcription initiation by holoenzyme [\(\alpha_2\beta\beta'\sigma\)] are discussed.

**Results**

**Amino acids within conserved region 1.1 of \(\sigma^{70}\) inhibit DNA binding**

The amino-terminal portion of \(\sigma\), called region 1, is divided into two conserved segments, termed 1.1 and 1.2 [Fig. 1A]. Region 1.1 is conserved only among the primary \(\sigma\)-factors, whereas region 1.2 is conserved among both primary and alternative \(\sigma\)-factors (except for \(\sigma^E\) and \(\sigma^S\)). Our previous experiments indicated that removing all of region 1 from \(\sigma^{70}\) [amino acids 1–130] was sufficient to permit specific and nonspecific binding of \(\sigma^{70}\) to DNA. To further define the role of the amino-terminal region of \(\sigma^{70}\) in controlling DNA-binding activity, we made deletions into both region 1 and the "spacer," a region unique to \(\sigma^{70}\) that follows region 1 [Fig. 1B]. The relative DNA-binding activity of each deleted protein [purified as glutathionine S-transferase (GST) fusion proteins] was assessed using nitrocellulose filter-binding assays [see Materials and methods], and the results are shown in Figure 1B. Removal of the first 50 amino acids of \(\sigma^{70}\) was sufficient to permit binding to DNA, indicating that the amino-terminal half of region 1.1 is necessary for inhibition of binding. Removing 180–273 amino acids from the amino terminus allowed truncated derivatives to bind DNA an order of magnitude more tightly, suggesting that amino acids in this region also play a role in modulating DNA binding.

The activity of the altered \(\sigma^{70}\) fusion proteins was also tested in vivo. With the exception of GST\(\sigma^{70}[9–613]\), none of the amino-terminal truncations or internal deletions was able to complement the temperature-sensitive defect of a mutant \(\sigma^{70}\) strain [\(\text{rpoD}285\)]. We assume that these deleted proteins fold properly because of their DNA-binding activity. This suggests that each deletion removes an essential portion of \(\sigma^{70}\). One of the noncomplementing deletions [GST\(\sigma^{70}[A100–130]\)] removes only region 1.2, providing evidence that this region is necessary for \(\sigma^{70}\) function. Interestingly, fusions lacking either the first 50 or 72 amino acids of \(\sigma^{70}\) were detrimental to cell growth under noninducing conditions and lethal upon overexpression [Fig. 1B]. These two derivatives may interfere with normal \(\sigma^{70}\) function in vivo, possibly by competing for binding to core RNA polymerase or by carrying out inappropriate transcription.

**Figure 1.** Characterization of GST\(\sigma^{70}\) fusion proteins. [A] Linear diagram of \(\sigma^{70}\). The most highly conserved blocks of amino acid sequence are numbered 1–4 [Lonetto et al. 1992]. Subregions are indicated by different patterns within each main region. Region 2 is implicated in recognition of the –10 promoter consensus. Region 4 contains a helix–turn–helix motif and is implicated in recognition of the –35 promoter consensus. [B] Function of amino-terminally altered GST\(\sigma^{70}\) fusion proteins. GST was fused to the amino terminus of several truncated \(\sigma^{70}\) derivatives. The amino acid sequences included are designated by the schematic diagram at right. The first column indicates the amino acids that remain, except for the bottom line where region 1.2 (amino acids 100–130) was deleted. The second column indicates the ability of the fusion, encoded by the pGEX plasmid, to complement the temperature-sensitive growth defect of the \(\text{rpoD}285\) [\(\text{rpoD}800\)] allele at 42°C in the absence of IPTG. The third column displays the effect of overexpression of each fusion protein on viability. The strain used for these experiments was XL-1. The fusions were encoded by the pGEX plasmid under the control of the ptac promoter. The lacI allele is present on the plasmid. Growth was at 37°C to mid-log phase, where IPTG was added to a final concentration of 0.1 mM. The fourth column summarizes the DNA-binding ability for each fusion. DNA-binding titrations, using \(3^P\)-labeled ptac promoter DNA, were conducted as described previously [Dombroski et al. 1992]. The amount of fusion protein required for half-maximal retention of protein–DNA complexes on nitrocellulose filters was in the range of 1–100 nM. Each plus sign (+) roughly represents an order of magnitude difference in binding affinity, where ++ is the strongest binding.
The amino-terminal inhibitory domain of σ^70 interacts with carboxy-terminal amino acids

We determined whether the amino-terminal region of σ^70 could inhibit DNA binding in trans. Several GST fusion proteins were constructed to carry only the amino-terminal sequences of E. coli σ^70 [Fig. 2A]. Each of these potential "inhibitors" was assayed for its ability to inhibit the DNA binding of GSTσ^70[360-613]. GSTσ^70[360-613] is a σ^70 fusion protein containing both the region 2 and region 4 DNA-binding domains and was shown previously to bind to DNA [Dombroski et al. 1992]. The three amino-terminal fusions containing 100 or more amino acids inhibited DNA binding in trans [Fig. 2B; data not shown]. An excess of the inhibitory protein was required for inhibition, to approximate the high local concentration of the amino terminus with respect to the carboxyl terminus in the intact protein. Although deletion of the first 50 amino acids is sufficient to relieve inhibition of DNA binding, an amino-terminal fusion carrying only the first 50 amino acids of σ^70 did not inhibit DNA binding in trans, even at a 50-fold molar excess. There are several plausible explanations. First, this small fusion protein may not fold correctly in vitro. Second, it may not be able to associate properly with the binding domain. Finally, the presence of GST may potentially interfere with normal function of this inhibitor.

The site of inhibition within σ^70 was investigated by determining whether the amino-terminal inhibitory domain affects the DNA binding of region 2 or region 4 in trans. GSTσ^70[506-613], carrying a portion of region 3 (14 amino acids) and the region 4 DNA-binding domain, was negatively affected by GSTσ^70[1-100], whereas GSTσ^70[372-466], comprised solely of the region 2 DNA-binding domain, was not [Fig. 2B]. These experiments suggest that the target of the amino-terminal regulatory region of σ^70 lies within the carboxy-terminal 107 amino acids and directly affects the DNA-binding domain of region 4.

If the amino-terminal inhibitory domain interacts solely with region 4, then GSTσ^70[8-506], which maintains DNA-binding domain 2.4 in cis to the amino-terminal inhibitory domain, should still bind DNA. However, this simple prediction is not borne out completely. This fusion still binds DNA but with about a 10-fold reduction in affinity [data not shown]. We consider this observation further in the discussion.

Binding of alternative σ-factors to DNA is controlled by their amino termini

The alternative σ-factors are required for transcription of specific sets of genes during various physiological or de-
velopmental states of the prokaryotic cell. Each alternative σ has a distinct amino terminus that is not conserved among σ-factors, raising the possibility that amino-terminal modulation of σ function may not be ubiquitous. As a preliminary assessment of the generality of such regulation, we examined the DNA-binding properties of three alternative σ-factors with and without their amino termini. These σ-factors lack any homology to the putative inhibitory domain of region 1.1 of σ70 [Lonetto et al. 1992], and each is distinctly different with respect to its amino-terminal composition [Fig. 3].

σ70 from Salmonella typhimurium, has a short 9-amino-acid block of nonconserved sequence at its amino terminus immediately preceding the first DNA-binding domain. It is rare among the bacterial σ-factors because it is devoid of sequences corresponding to region 1.2 as well as 1.1. Full-length σ70 fused to GST bound to plfIC promoter DNA (data not shown), but the magnitude of binding was ~10-fold lower than for the analogous σ70 fusion. Deletion of the first 9 amino acids of nonconserved sequence did not improve binding ability. Thus, σ70 is not amino-terminally controlled for DNA binding.

σK from Bacillus subtilis has a 20-amino-acid amino-terminal extension immediately preceding conserved regions 1.2–4. This extension comprises a "pro" sequence that is cleaved in a developmentally regulated manner to generate active σK [Lu et al. 1990]. Two derivatives of B. subtilis σK were characterized for DNA binding and amino-terminal regulation: GSTσK[Δpro] deleted for the 20-amino-acid pro sequence and GST σK[28–221] deleted for both the pro sequence and the first 27 amino acids, including region 1.2 [Fig. 4A]. While full-length σK bound to DNA, deletions of either the pro sequence alone or the pro sequence plus region 1.2 resulted in an order of magnitude increase in tightness of binding [Fig. 4B]. In this case, it appears that the amino-terminal pro sequence is acting to modulate the DNA-binding activity of σK but places less severe restrictions on binding than does the amino-terminal inhibitory domain of σ70.

Deleting the amino-terminal amino acid sequences of σK also had profound effects on the stability of the poly-peptides. GSTσK was detrimental to E. coli growth and was less stable than its σ70 counterparts. GSTσK[Δpro] and GSTσK[28–221] were progressively less stable in vivo, in agreement with studies of B. subtilis σK, for which deletion of the pro sequence and other amino-terminal amino acids alters protein stability in B. subtilis [Peters et al. 1992].

σ32, the heat shock σ-factor in E. coli, has 18 amino acids of nonconserved sequence at its amino terminus, which is followed by homology to σ70 in conserved regions 1.2, 2, 3, and 4. This σ is more closely related to σ70 than the other alternative σ-factors tested [Lonetto et al. 1992]. Full-length σ32, free or fused to GST, was unable to bind to pgroE promoter DNA. However, deletion of the first 46 amino acids, which removes both the nonconserved amino-terminal sequences and region 1.2, allowed σ32 to bind DNA [Fig. 5B]. Thus, in analogy to σ70, the ability of σ32 to bind to DNA is tightly controlled by...
an amino-terminal inhibitory region. We attempted to further define the inhibitory region by constructing GSTσ32[19–284], which leaves region 1.2 intact. However, this fusion was so unstable that we were unable to recover any intact protein, even from protease-deficient strains of E. coli.

All three GSTσ32 fusions were tested for their ability to complement rpoH165, an allele encoding a temperature-sensitive σ32. Both GSTσ32 and GSTσ32[19–284] restored growth at high temperature, whereas GSTσ32[47–284] did not. In addition, GSTσ32 and GSTσ32[19–284] both restored transcription from σ32 promoters in a strain deleted for σ32. These results suggest that the first 18 amino acids of σ32 are dispensable but that region 1.2, contained between amino acids 18 and 46, is necessary for some aspect of σ32 function in vivo.

All of the various derivatives of the three alternative σ-factors were characterized for DNA-binding specificity using equilibrium competition analysis as described previously (Dombroski et al. 1992). In all cases, specificity for promoter over nonpromoter DNA was in the range of two- to fivefold (data not shown). Thus, as seen for σ70, the majority of binding that we observe is attributable to nonspecific interactions between σ and DNA.

The amino-terminal inhibitory domain of σ70 inhibits σ32

The specificity of inhibition by region 1.1 of σ70 was addressed by assessing the effect of GSTσ70[1–100] in trans on the binding of σ32, σK, and σF to DNA (Fig. 6A). No inhibition was observed for GSTσK or GSTσF[28–221], as shown in Figure 6B, even at 50-fold molar excess of inhibitor over binding protein. However, a striking effect was observed for GSTσ32[47–284]. The magnitude of inhibition for this σ32 derivative was very similar to that seen with the analogous σ70 fusion, GSTσ70[360–613] (see Fig. 2B). These results indicate that the amino-terminal amino acids of σ70 and σ32 are able to associate with the carboxy-terminal amino acids of either σ70 or σ32 and act to mask or inhibit DNA binding. The mechanism for amino-terminal modulation of σ32 may be quite similar to σ70 despite the lack of amino acid homology in their respective inhibitory domains.

Several mutations that occur in the helix–turn–helix of region 4 alter the promoter recognition phenotypes of holoenzyme. An arginine to phenylalanine substitution at position 588 of σ70 affects recognition of a particular base pair in the –35 consensus (Dombroski et al. 1992). Arg-588 is conserved in σ70 and σ32, both of which are inhibited by the amino terminus of σ70, whereas neither σK nor σF carry an Arg at the equivalent position (Lonetto et al. 1992), and neither is inhibited. Thus, we tested whether Arg-588 was involved in the inhibition of binding seen for σ70 and σ32. DNA-binding fusions were constructed corresponding to GSTσ70[420], carrying amino acids 420–613 (Dombroski et al. 1992), with either wild type region 4 or the RF588 change in region 4. Both were inhibited to an equivalent extent by the amino-terminal 100 amino acids of σ70 (data not shown). It appears that the occurrence or absence of inhibition cannot be explained simply by this single amino acid difference.

Discussion

σ-Factors have long been recognized as the specificity subunit for interactions between RNA polymerase and promoter DNA, but the mechanism by which σ confers promoter specificity has only begun to be understood. Genetic experiments initially provided evidence for interaction between σ and the –10 and –35 consensus hexamers of promoters (Gardella et al. 1989; Siegol et al. 1989; Zuber et al. 1989; Daniels et al. 1990; Waldburger et al. 1990). Direct physical contact between σ and promoter DNA, however, could not be observed. This paradox was resolved by the demonstration that
truncation of σ70 from the amino terminus generates polypeptides that are now capable of promoter-specific DNA recognition and binding suggesting that the amino-terminal amino acids act to inhibit DNA binding (Dombroski et al. 1992). A model was proposed whereby σ70 undergoes an allosteric interaction with core RNA polymerase, which exposes its DNA-binding domains and allows holoenzyme to engage in specific transcriptional activity [Fig. 7]. Here, we report that the minimal amino-terminal inhibitory domain of σ70 resides within conserved region 1.1. Inhibition of binding can also be observed in trans and requires between 50 and 100 amino acids from the amino terminus. The fact that the isolated region 4 DNA-binding domain is sensitive to inhibition, whereas the isolated region 2 domain is not, suggests that region 4 may serve as the primary site for interaction with the amino-terminal inhibitory domain. Additionally, we show that several alternative σ-factors are able to bind to DNA and that, in some cases, binding is affected by the amino-terminal domains of these σ-factors. From these results we propose a general mechanism for modulation of σ-factor activity, where the amino-

Figure 7. Model for σ70 function. Free full-length σ70 is shown folded into a conformation, where the amino-terminal inhibitory domain is positioned such that it blocks the carboxy-terminal DNA-binding domains. We propose that upon interaction with the core subunits to generate holoenzyme, σ70 undergoes a conformational rearrangement that exposes the DNA-binding domains and permits sequence-specific promoter recognition and transcription initiation.
terminal domain is responsible for determining ability to recognize and bind to promoter DNA and can contribute to the stability of certain σ-factors in vivo.

Amino-terminal inhibition of DNA binding

The primary σ-factors possess a novel region of homology from amino acids 1–100 called region 1.1. The DNA-binding characteristics of a progressive deletion series from the amino terminus of σ70 indicate that within the first 50 amino acids lies an inhibitory domain, which prevents DNA binding by the carboxy-terminal DNA-binding domains. The alternative σ-factors, which are required for more specialized transcriptional control, have no amino-terminal homology to region 1.1 of σ70, prompting us to ask whether these σ-factors can bind to DNA. Three distinct alternative σ-factors, from three different organisms were included in this study. Each of these σ-factors controls a specific response or developmental pathway. The heat shock σ from E. coli, σ32, is responsible for transcription of the heat shock genes upon temperature upshift. σ32 shares a great degree of similarity to σ70, with the exception of its amino terminus. It lacks region 1.1 but has an 18-amino-acid extension of nonconserved sequence. Surprisingly, our results show that full-length σ32 cannot bind to DNA even though it lacks a σ70-like inhibitory domain. Deletion of the first 46 amino acids allows DNA binding at a level equivalent to the σ70 homolog GSTσ70[360–613], suggesting that σ32 contains its own amino-terminal inhibitory domain. The amino terminus of σ32 is unrelated in primary sequence to the inhibitory domain of σ70, but binding of σ32 to DNA appears to be controlled by a qualitatively similar mechanism.

σK from B. subtilis is required for expression of mother-cell-specific genes during the developmental cascade of events leading to sporulation. σK is unusual because it belongs to a small group of σ-factors that is synthesized with an amino-terminal pro-sequence and must be processed to activate transcription [LaBell et al. 1987; Kroos et al. 1989; Straiger et al. 1989]. Pro-σK possesses a 20-amino-acid amino-terminal extension that lacks homology to any other σ proteins. Although pro-σK is inactive in vivo, its fusion protein derivative GSTσK binds to DNA in vitro. Removal of the pro-sequence improves binding by an order of magnitude. Thus, the ability of σK to bind DNA is modulated, but not tightly controlled, by its amino-terminal domain. This increase in affinity for DNA may reflect the requirement for removal of the pro-sequence in vivo to activate σK. The discovery that the pro-sequence of σK governs DNA binding fits with studies conducted with the related σE. LaBell et al. [1987] found that pro-σE associates with core RNA polymerase but is unable to initiate transcription until the pro-sequence is removed.

In S. typhimurium, the product of the flaA gene, σF, is utilized by RNA polymerase for the transcription of flagellin and all other late genes in the complex transcriptional cascade of events resulting in flagellar biosynthesis [Helmann 1991]. σF completely lacks sequences corresponding to regions 1.1 or 1.2 in the σ70 family. As expected, GSTσF was able to bind to DNA because it is devoid of a significant amino-terminal extension. Although the binding was weaker than for most other σ-factors, tightness of binding to promoter DNA may be an element of the mechanism for control of the flagellar operons.

In summary, both the primary and alternative σ-factors possess an inherent DNA-binding activity. This activity, however, cannot be observed under all conditions or states of the protein. Our results argue that the interaction between several different σ-factors and DNA can be regulated at the level of protein structure and/or conformation. The amino-terminal extensions of these σ-factors provide the means for inhibition or modulation through interactions with carboxy-terminal portions of the polypeptide. The details of these interactions are likely to be specific for each different σ.

Mechanism of inhibition

The target site for the inhibitory action of the amino terminus of σ70 may be the DNA-binding domain of region 4. Inhibition of binding in trans by region 1.1 was observed for fusions containing either regions 2–4 [both DNA-binding domains] or region 4 [including the carboxy-terminal 14 amino acids of region 3], but not region 2 alone. Because our model predicts that full-length σK undergoes a conformational change upon association with core to expose its DNA-binding domains, it follows from these experiments that region 1.1 is directly involved in conformational adjustments that affect DNA recognition and binding by the region 4 DNA-binding domain. This proposal must be qualified by the observation that a fusion protein consisting of amino acids 8–506 [regions 1, 2, and two-thirds of region 3] does not bind to DNA as well as region 2 alone does. This introduces the possibility that region 1 can also affect region 2 when it is in the context of its normal flanking sequences. Possibly, the inhibitory effect on region 2 is mediated by an allosteric change that must be communicated in cis, rather than by direct binding. Inhibition of binding by the amino terminus for full-length σ may be more complicated than predicted simply by experiments that demonstrate inhibition of binding in trans.

At present, we do not know the target for amino-terminal inhibition in region 4. One possibility is that the amino-terminal inhibitory region recognizes amino acids involved in base-specific contacts with the promoter. The fact that the amino-terminal inhibitory domain of σ70 functions to effectively inhibit DNA binding in trans by regions 2–4 of σ32 would be in accord with this idea, as the recognition helices of the two σ-factors differ by only a single amino acid. Lack of inhibition of σK and σF might reflect less homology in the recognition helix. However, the fact that the arginine to phenylalanine substitution at position 588 in the recognition helix does not relieve amino-terminal inhibition argues against, but does not rule out, this idea.

It is currently unclear whether RNA polymerase rec-
ognizes the −35 and −10 regions of the promoter simultaneously or sequentially. Footprinting studies on putative intermediates in transcription obtained at low temperature favor a sequential model, with initial recognition in the −35 region of the promoter (Kovacic 1987; Cowing et al. 1989; Mccas et al. 1991). Some studies of mutant promoters are also in accord with the idea that initial recognition resides solely in −35 contacts (Hawley and McClure 1980, 1982; Shih and Gussin 1983); however, other studies contradict this idea (Stefano and Gralla 1982, Szoke et al. 1987). Our data can be interpreted to favor the sequential mechanism. Because region 4 is responsible for −35 contacts, and if −35 contacts represent the earliest stages of promoter binding, then primary inhibition of binding at the region 4 DNA-binding domain would be a straightforward approach to modulating σ-factor activity.

Many examples of amino-terminally self-regulated proteins have been cited in both prokaryotic and eukaryotic organisms. In B. subtilis, the activation function of the SpoOA transcription factor is inhibited by its amino-terminal domain (Ireton et al. 1993). Likewise, the amino-terminal domain of the Fix transcriptional activator from Rhizobium meliloti negatively regulates its activation function (Kahn and Ditta 1991). The CheB methylase is regulated in the same manner (Simms et al. 1985). In the case of the luxR activator of Vibrio fischeri, the amino-terminal domain masks the activation function of the carboxy-terminal domain unless an autoinducer is present (Choi and Greenberg 1991). The masking of a functional domain by an accessory domain has also been documented for the glucocorticoid receptor where in the absence of hormone, the steroid hormone binding domain inhibits the transcriptional activation domain (Godowski et al. 1987; Hollenberg et al. 1987). In conclusion, the σ-factors likely represent another set of members for this growing family of intramolecularly regulated macromolecules, where a conformational change must be induced to relieve the effect of the inhibitory domain.

Materials and methods

Materials

Restriction endonucleases, T4 DNA ligase, and T4 polymerase was from Perkin-Elmer. [γ−32P]ATP (3000 Ci/mmole) was purchased from New England Nuclear or Amer sham. Oligonucleotide filter disks (BA85) were from Schleicher & Schuell. Isopr opyl-β-D-thiogalactoside was obtained from Sigma. Buffer components were from Mallinkrodt, Sigma, or Fisher Scientific. Oligonucleotides were synthesized by Genosys.

Plasmid constructions

The parent plasmid for all fusions of σ to GST was pGEX-2T (Smith and Johnson 1988). Expression of the fusion proteins is under the control of the IPTG-inducible ptac promoter. The lacI allele is present on the same plasmid. Insert DNA, carrying specific segments of rpoD(¢7°), rpoH(¢21), fliA(¢2), or sigK(¢5), was generated using the polymerase chain reaction (PCR). PCR reactions contained 100 mM Tris-HCl (pH 8.3), 500 mM KC1, 15 mM MgCl2, 0.4 mM dNTPs, 50 pmole of each primer, 50 ng of template DNA, and 2.5 units of Amplitaq DNA polymerase. A Coy TempCycler was programmed for 35 cycles employing 95°C for denaturation, 50–60°C for annealing, and 75°C for extension with a time of 1 min for each segment. Oligonucleotides (27–30 nucleotides in length) corresponding to the 5’ and 3’ ends of these segments were designed to amplify the region of interest and to incorporate BamHI and EcoRI restriction sites, respectively. pHH62 (Hu and Gross 1988) was the template DNA for the rpoD fusions, whereas pKH439 (gift of K. Hughes, University of Washington, Seattle) and pSK5 (Lu et al. 1991) were the templates for the fliA and sigK fusions. DNA fragments were purified by elution from agarose gels (Sambrook et al. 1989) and ligated (Maniatis et al. 1992) into the pGEX-2T vector to form an in-frame fusion. One internal deletion of σ¢70 was created by synthesizing two PCR fragments where the amino terminus of the protein was encoded by a fragment with a 5’ BamHI site and a 3′ XbaI site while the carboxyl terminus was encoded by a fragment with a 5′ XbaI site and a 3′ EcoRI site. These two fragments were then ligated into the BamHI and EcoRI sites of pGEX-2T in a single step. This construction results in insertion of a serine and arginine between amino acids 100 and 130 of σ¢70. pGEX–σ¢8[Δ8, Δ506–613] was constructed by digesting pGEX–σ¢8[8] (Dombroski et al. 1992) with NcoI to drop out region 4 and religating.

Complementary oligonucleotides, corresponding to the fliC promoter were synthesized to a length of 50 nucleotides, annealed to form double-stranded DNA, digested with EcoRI and HindIII, and ligated into the EcoRI and HindIII sites of M13mp19. pAD100 carries the groE promoter, from −60 to +10, upstream of the galk gene. The groE promoter is derived from pSC146 (gift of L. Kroos, Michigan State University, East Lansing). These plasmids served as PCR templates to generate DNA substrates for nitrocellulose filter binding.

Overproduction and purification of fusion proteins

Most of the fusion proteins were purified from the E. coli strain XL-1 (Stratagene); however GST¢7¢221 and GST¢32¢100–130 50 were sufficiently unstable in this strain that a protease-deficient strain, BL21 (ompT, lon) (Novagen, Inc.), was required to obtain reasonable amounts of intact protein. Expression of each GST–σ fusion protein was induced with 0.1 mM IPTG at 37°C for 3–4 hr. Affinity purification using glutathione–agarose beads [sulfur linkage, Sigma] has been described in detail elsewhere (Dombroski et al. 1992).

Nitrocellulose filter-binding assays

The 38P-labeled DNA fragments used as substrates in binding assays were synthesized using PCR amplification as described previously (Dombroski et al. 1992). The binding of various σ derivatives was measured by determining the retention of protein–DNA complexes on nitrocellulose filters. These experiments were performed exactly as described previously (Dombroski et al. 1992).

Inhibition of binding in trans was executed by first mixing the inhibitor protein with the binding protein on ice and incubating for at least 5 min. Proteins were previously diluted appropriately in protein dilution buffer [10 mM Tris-HCl (pH 8), 10 mM KC1, 10 mM β-mercaptoethanol, 1 mM EDTA, 0.1% Triton X-100, 0.4 μg/ml of BSA]. Extended incubation periods did not affect the inhibition results. Nitrocellulose filter-binding assays
were then performed as described above. All data represent the average of two or more duplicate experiments.

Complementation of defective σ alleles

The ability of the fusion proteins to rescue σ mutant phenotypes was assessed by transforming the pGEX derivatives of σ<sup>70</sup> into P90A5c (Calendar et al. 1988), which carries the temperature-sensitive rpoD285 allele (Harris et al. 1978). rpoD285 is identical to rpoD800, as determined by sequence analysis. The transformants were grown at 30°C, 37°C, and 42°C in the absence of IPTG and scored for complementation of the temperature-sensitive growth phenotype. pGEX derivatives of σ<sup>32</sup> were transformed into ML20035, R4 derivative carrying a deletion of the rpoH allele (Kusukawa and Yura 1988), as well as a λ prophage carrying the σ<sup>32</sup> promoter (pPH) (Cowin et al. 1985) upstream of lacZ. Transformants were scored for color phenotype on X-gal indicator plates. Additionally, the σ<sup>32</sup> derivatives were tested in the strain CAG597 (temperature-sensitive), encoding a temperature-sensitive suppressor tRNA and the rpoH165 amber mutation (Neidhardt et al. 1984). The resulting strains were grown at 30°C, 37°C, and 42°C in the absence of IPTG and scored for complementation of the temperature-sensitive growth phenotype.

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References

Burgess, R.R. and A.A. Travers. 1970. <i>Escherichia coli</i> RNA polymerase: Purification, subunit structure, and factor requirements. <i>Fed. Proc.</i> 29: 1164–1169.

Burgess, R.R., A.A. Travers, J.J. Dunn, and E.K.F. Bautz. 1969. Factor stimulating transcription by RNA polymerase. <i>Nature</i> 221: 43–44.

Calendar, R., J.W. Erickson, C. Halling, and A. Nolte. 1988. Deletion and insertion mutations in the rpoH gene of <i>Escherichia coli</i> that produce functional σ<sup>32</sup>. <i>J. Bacteriol.</i> 170: 3479–3484.

Choi, S.H. and E.P. Greenberg. 1991. The C-terminal region of the Vibrio fischeri LuxR protein contains an inducer-independent lux gene activating domain. <i>Proc. Natl. Acad. Sci.</i> 88: 11115–11119.

Cowin, D.W., J.C.A. Bardwell, E.A. Craig, C. Woolford, R. Hendrix, and C.A. Gross. 1985. Consensus sequence for <i>Escherichia coli</i> heat shock promoter genes. <i>Proc. Natl. Acad. Sci.</i> 82: 2679–2683.

Cowin, D.W., J. Mecsas, M.T. Record Jr., and C.A. Gross. 1989. Intermediates in the formation of the open complex by RNA polymerase holoenzyme containing the sigma factor σ<sup>87</sup> at the groE promoter. <i>J. Mol. Biol.</i> 210: 521–530.

Daniels, D., R. Zuber, and R. Losick. 1990. Two amino acids in an RNA polymerase σ factor involved in the recognition of adjacent base pairs in the –10 region of a cognate promoter. <i>Proc. Natl. Acad. Sci.</i> 87: 8075–8079.

Dombroski, A.J., W.A. Walter, M.T. Record Jr., D.A. Siegel, and C.A. Gross. 1992. Polypeptides containing highly conserved regions of transcription initiation factor σ<sup>70</sup> exhibit specificity of binding to promoter DNA. <i>Cell</i> 70: 501–512.

Gardella, T., H. Moyle, and M.M. Suskind. 1989. A mutant <i>Escherichia coli</i> σ<sup>70</sup> subunit of RNA polymerase with altered promoter specificity. <i>J. Mol. Biol.</i> 206: 579–590.

Godowski, P.J., S. Rusconi, R. Miesfeld, and K.R. Yamamoto. 1987. Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement. <i>Nature</i> 325: 365–368.

Gribskov, M. and R.R. Burgess. 1986. Sigma factors from <i>E. coli</i>, <i>B. subtilis</i>, phage SP01, and phage T4 are homologous proteins. <i>Nucleic Acids Res.</i> 14: 6745–6763.

Gross, C.A., M. Lonetto, and R. Losick. 1992. Sigma factors in transcriptional regulation (ed. S.L. McKnight and K.R. Yamamoto), vol. 1, pp. 129–176. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Harris, J.D., J.S. Heilig, I.I. Martinez, R. Calendar, and L.A. Isaksen. 1978. Temperature sensitive Escherichia coli mutant producing a temperature-sensitive σ subunit of DNA-dependent RNA polymerase. <i>Proc. Natl. Acad. Sci.</i> 75: 6177–6181.

Hawley, D.K. and W.R. McClure. 1980. In vitro comparison of initiation properties of bacteriophage λ wild-type P<sub>4</sub> and x4 mutant promoters. <i>Proc. Natl. Acad. Sci.</i> 77: 6381–6385.

———. 1982. Mechanism of activation of transcription initiation from the A<sub>PR</sub> promoter. <i>J. Mol. Biol.</i> 157: 493–525.

Helmann, J.D. 1991. Alternative sigma factors and the regulation of flagellar gene expression. <i>Mol. Microbiol.</i> 5: 2875–2882.

Helmann, J.D. and M.J. Chamberlin. 1988. Structure and function of bacterial sigma factors. <i>Annu. Rev. Biochem.</i> 57: 839–872.

Hollenberg, S.M., V. Giguere, P. Segui, and R.M. Evans. 1987 Colocalization of DNA-binding and transcriptional activation functions in the human glucocorticoid receptor. <i>Cell</i> 49: 39–46.

Hu, J.C. and C.A. Gross. 1988. Mutations in rpoD that increase expression of genes in the mal regulon of <i>Escherichia coli</i> k-12. <i>J. Mol. Biol.</i> 203: 15–27.

Ireton, K., D.Z. Rudner, K.J. Siranosian, and A.D. Grossman. 1993. Integration of multiple developmental signals in <i>Bacillus subtilis</i> through the Spo0A transcription factor. <i>Genes & Dev.</i> 7: 283–294.

Kahn, D. and G. Ditta. 1991. Molecular structure of FixL: Homology of the transcriptional activator domain with the –35 binding domain of sigma factors. <i>Mol. Microbiol.</i> 5: 987–997.

Kovacic, R.T. 1987. The closed complexes between <i>Escherichia coli</i> RNA polymerase and two promoters, T7-A3 and lacUVS. <i>J. Biol. Chem.</i> 262: 13654–13661.

Kroos, L., B. Kunkel, and R. Losick. 1989. Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. <i>Science</i> 243: 526–529.

Kudo, T. and R.H. Doi. 1981. Free σ factor of <i>Escherichia coli</i> RNA polymerase can bind to DNA. <i>J. Biol. Chem.</i> 256: 9778–9781.

Kudo, T., D. Jaffe, and R.H. Doi. 1981. Free sigma subunit of <i>Bacillus subtilis</i> RNA polymerase binds to DNA. <i>Mol. Gen. Genet.</i> 181: 63–68.

Kusukawa, N. and T. Yura. 1988. Heat shock protein GroE of <i>Escherichia coli</i>: Key protective roles against thermal stress. <i>Genes & Dev.</i> 2: 879–882.
LaBell, T.L., J.E. Trempy, and W.G. Haldenwang. 1987. Sporulation-specific σ factor σ^29 of Bacillus subtilis is synthesized from a precursor protein, P^31. Proc. Natl. Acad. Sci. 84: 1784–1788.

Lonetto, M., M. Gribskov, and C. Gross. 1992. MiniReview: The σ^70 family: Sequence conservation and evolutionary relationships. J. Bacteriol. 174: 3843–3849.

Losick, R. and J. Pero. 1981. Cascades of sigma factors. Cell 25: 582–584.

Lu, S., R. Halberg, and L. Kroos. 1990. Processing of the mother-cell σ factor σ^54 may depend on events occurring in the forespore during Bacillus subtilis development. Proc. Natl. Acad. Sci. 87: 9722–9726.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Mecsas, J., D.W. Cowing, and C.A. Gross. 1991. Development of RNA polymerase-promoter contacts during open complex formation. J. Mol. Biol. 220: 585–597.

Neidhardt, F.C., R.A. Van Bogelen, and V. Vaughn. 1984. Genetics and regulation of heat shock promoters. Annu. Rev. Genet. 18: 295–329.

Peters H.K. III, H.C. Carlson, and W.G. Haldenwang. 1992. Mutational analysis of the precursor-specific region of Bacillus subtilis σ^54. J. Bacteriol. 174: 4629–4637.

Ramesh, U. and C.F. Meares. 1989. Footprint of the sigma protein. Biochem. Biophys. Res. Commun. 160: 121–125.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shih, M.-C. and G.N. Gussin. 1983. Mutations affecting two different steps in transcription initiation at the phage λ PrM promoter. Proc. Natl. Acad. Sci. 80: 496–500.

Siegel, D.A., J.C. Hu, W.A. Walter, and C.A. Gross. 1989. Altered promoter recognition by mutant forms of the σ^70 subunit of Escherichia coli RNA polymerase. J. Mol. Biol. 206: 591–603.

Simms, S.A., M.G. Keane, and I. Stuck. 1985. Multiple forms of the CheB methyltransferase in bacterial sensing. J. Biol. Chem. 260: 10161–10168.

Smith, D.B. and K.S. Johnson. 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione-S-transferase. Gene 67: 31–40.

Stelano, J.E. and J.D. Gralla. 1982. Mutation-induced changes in RNA polymerase-lac p^5 promoter interactions. J. Biol. Chem. 257: 13924–13929.

Stragier, P., C. Parsot, and J. Bouvier. 1985. Two functional domains conserved in major and alternate bacterial sigma factors. FEBS Lett. 187: 11–15.

Stragier, P., B. Kunkel, L. Kroos, and R. Losick. 1989. Chromosomal rearrangement generating a composite gene for a developmental sigma factor. Science 243: 507–512.

Szoke, P.A., T.L. Allen, and P.L. DeHaseth. 1987. Promoter recognition by Escherichia coli RNA polymerase: Effects of base substitutions in the −10 and −35 regions. Biochemistry 26: 6188–6194.

Waldburger, C., T. Gardella, R. Wong, and M.M. Susskind. 1990. Changes in conserved region 2 of Escherichia coli σ^70 affecting promoter recognition. J. Mol. Biol. 215: 267–276.

Wellman, A. and C.F. Meares. 1991. Footprint of the sigma protein: A re-examination. Biochem. Biophys. Res. Commun. 177: 140–144.

Wu, C.-W., L.R. Yarbrough, Z. Hillel, and F.Y.-H. Wu. 1975. Sigma cycle during in vitro transcription: Demonstration by nanosecond fluorescence depolarization spectroscopy. Proc. Natl. Acad. Sci. 72: 3019–3023.

Zillig, W., K. Zechel, D. Rabussay, M. Schachner, V.S. Sethi, P. Palm, A. Heil, and W. Seifert. 1970. On the role of different subunits of DNA-dependent RNA polymerase from E. coli in the transcription process. Cold Spring Harbor Symp. Quant. Biol. 35: 47–58.

Zuber, P., J. Healy, H.L. Carter III, S. Cutting, C.P. Moran Jr., and R. Losick. 1989. Mutation changing the specificity of an RNA polymerase σ^70 factor. J. Mol. Biol. 206: 605–614.
Amino-terminal amino acids modulate sigma-factor DNA-binding activity.

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References

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