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Mini-review

Role of the RNA polymerase sigma subunit in transcription initiation

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Abstract

In bacteria, σ subunits direct the catalytically competent RNA polymerase core enzyme to promoters. Recent advances in our understanding of bacterial RNA polymerase reveal that σ subunits are intimately involved in all aspects of transcription initiation including promoter location, promoter melting, initiation of RNA synthesis, abortive initiation and promoter escape.

Keywords: RNA polymerase; Sigma subunits; Transcription; Initiation

DNA-dependent RNA polymerase (RNAP) is the central enzyme of gene expression and a major target for regulation. Core RNAP from *Escherichia coli*, the enzyme which is the best-studied functionally, contains five polypeptides: β′ (1407 amino acids), β (1342 amino acids), a dimer of α (329 amino acids) and the ω subunit (91 amino acids). Most other bacterial RNAPs have identical subunit composition. Core RNAPs from eukaryotes and archaea also share the basic $\alpha_2\beta\beta'\omega$ subunit composition but contain 5–8 additional subunits that are absent from bacterial enzymes. The two largest subunits comprise ∼ 60% of RNAP mass and appear to be principally responsible for most of the enzyme's catalytic functions.

The structures of core RNAPs from thermophilic eubacterium *Thermus aquaticus* and yeast RNAP II have been determined and both structures are very similar to each other [9,29]. The molecules have a characteristic crab-claw shape, with a deep channel separating the jaws of the claw. The width of the channel is appropriate to accommodate the double-stranded DNA template. The catalytic Mg^{2+} ion is located deep within the channel. One jaw of the claw is composed of the largest (β′) subunit and the other is mostly composed of the second-largest (β) subunit. The α subunits are located outside of the channel at the back of the claw and serve to keep the two largest subunits together. The ω subunit is wound around the C-terminus of β′ and stabilizes its association with the $\alpha_2\beta$ subassembly [16].

The functional cycle of RNAP consists of transcription initiation, processive transcription elongation, and transcription termination. Transcription initiation, which can be subdivided into promoter complex formation, abortive initiation, and promoter escape, is the most heavily regulated stage of the transcription cycle.

RNAP core is catalytically proficient (i.e., is able to synthesize, in the presence of nucleoside triphosphate substrates, an RNA copy from a DNA template) but is unable to initiate transcription from promoters. In bacteria, binding of one of the several specificity σ subunits results in the formation of RNAP holoenzyme which can recognize promoters and initiate transcription. While the catalytic mechanism of RNAP is undoubtedly conserved between prokaryal, eukaryal, and archeal RNAPs, the mechanism of transcription initiation is not. Both eukaryotes and archaea rely on proteins and protein complexes unrelated to bacterial σ factors to recruit RNAP core to promoters.

Sequence comparisons reveal two unrelated families of bacterial sigma factors. Members of the first family of σ factors form RNAP holoenzymes that recognize promoters and form transcriptionally competent promoter complexes in the absence of other factors or energy sources. This family, which includes most bacterial σ factors is named after the prototypical housekeeping σ of *E. coli*, σ^{70}, and is the focus of this review. Members of the second,

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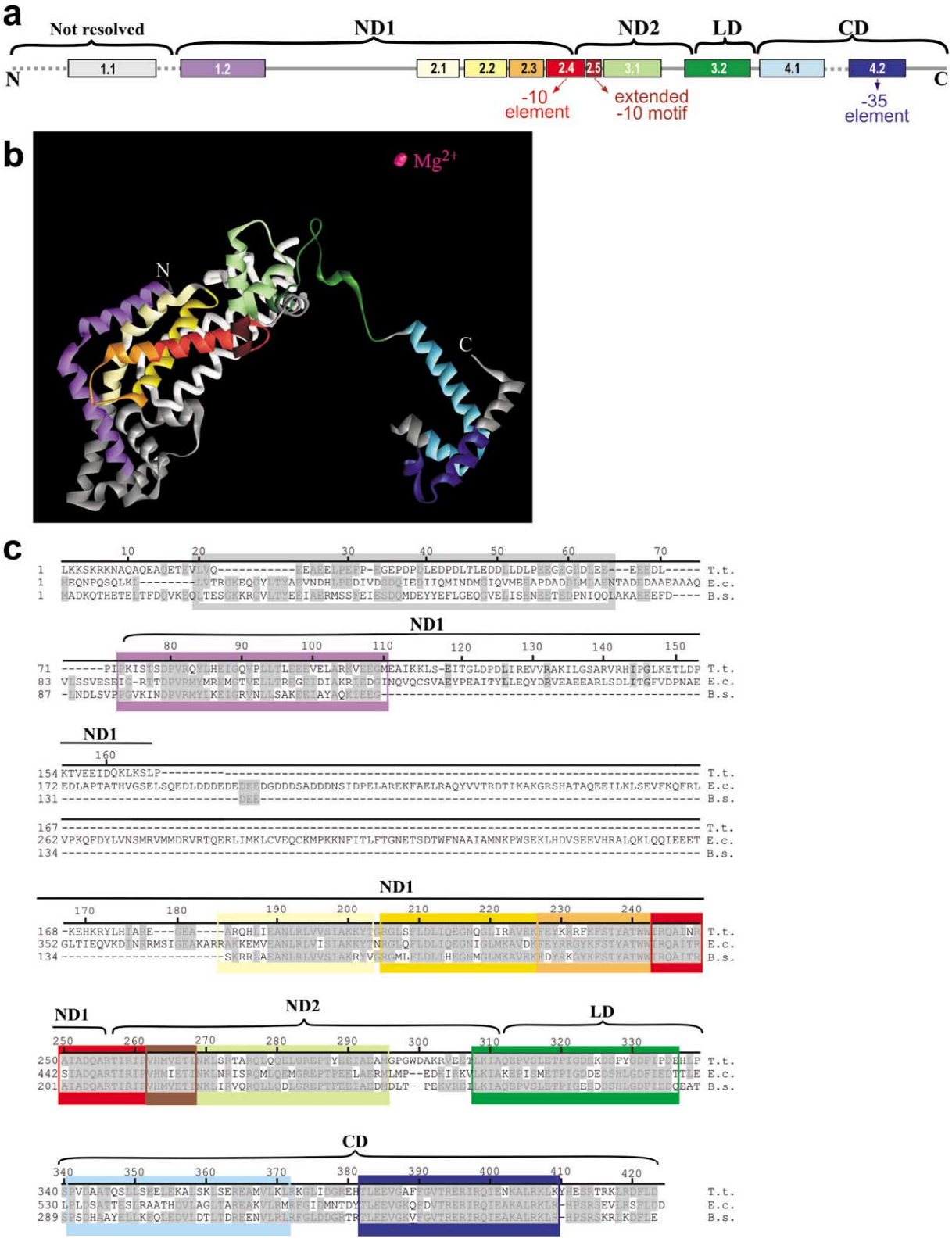

Fig. 1. Structure and amino acid sequence conservation of σ factors (after Ref. [25]). (a) Schematic representation of σ structural domains and evolutionarily conserved regions. The linear structure represents a σ^{70}-like subunit. Evolutionarily conserved regions are represented by numbered boxes and are color-coded. Structural domains are indicated above. Below, the interactions made by DNA binding domains of σ are indicated. (b) Ribbon diagram of σ^{A} factor from *T. thermophilus* RNAP holoenzyme structure [25]. The color coding is as in (a). The β′ subunit coiled-coil domain is shown in white. The positions of RNAP active site coincides with the positions of catalytic Mg^{2+} ions (magenta spheres). (c) Sequence alignments of *T. thermophilus* σ^{A}, *E. coli* σ^{70} and *B. subtilis* σ^{A}. The numbers above the alignment correspond to *T. thermophilus* σ^{A} amino acid positions. Dashes indicate gaps. Structural domains are indicated above the alignment. Regions of primary sequence conservation are indicated with the same color code as in (a) and (b).

minor family of σ factors, the σ^{54} family, form RNAP holoenzymes that recognize promoters but require additional protein factors and a source of energy in the form of ATP or GTP hydrolysis for formation of transcriptionally competent promoter complexes. The σ^{54} family has been reviewed elsewhere [4].

Multiple sequence alignments of proteins of the σ^{70} family reveal four regions of evolutionary conservation, termed regions 1 to 4, which can be further subdivided (Fig. 1a and 1c, Ref. [12]). Recent structural data show that proteins of the σ^{70} family consist of at least three α-helical domains that are connected to each other by highly flexible linkers (Fig. 1b). Such structural organization allows multiple conformations of σ to exist. Different biochemical properties of σ in its free state and in the context of RNAP holoenzyme are due to changes in the relative positions of σ structural domains, rather than to changes within the structural domains themselves. Evolutionarily conserved regions roughly coincide with σ structural domains. Evolutionarily conserved regions are color-coded in the alignment and the structural model of *T. thermophilus* σ^{A} presented in Fig. 1. The N-terminal region 1.1 is present in only some σ factors and is either disordered or mobile in both free σ and RNAP holoenzyme. Regions 1.2 (purple), 2.1 (light-yellow), 2.2 (yellow), 2.3 (orange) and 2.4 (red) jointly form a single structural domain, N-terminal domain 1 or ND1 [7,13,18,25]. The size of this domain varies between different σ factors due to the presence of evolutionarily variable sequence between regions 1.2 and 2.1 (gray, see also Fig. 1c). However, the relative positions of structural features formed by evolutionarily conserved regions 1.2, 2.1, 2.2, 2.3 and 2.4 are virtually identical in free proteolytic fragments of *E. coli* σ^{70} [13] and *T. aquaticus* σ^{A} [7], and in *T. aquaticus* and *T. thermophilus* σ^{A} bound to their respective RNAP core enzymes [17,24]. Regions 2.5 (dark-red) and 3.1 (light-green) form a small independent domain, ND2, that is connected by a short flexible tether to ND1 [7,18,25]. Region 3.2 (dark-green) forms a long linker domain (LD, Fig. 1) that is unstructured and proteolytically sensitive in free σ [23] but is fixed in the holoenzyme structure [18,25]. The C-terminal structural domain, CD, is formed by σ regions 4.1 (light-blue) and 4.2 (dark-blue) [7,18,25].

Though σ subunits are clearly responsible for promoter recognition by RNAP holoenzyme, multiple switches appear to have been built into these proteins to ensure that free σ subunits do not bind and occlude promoter DNA in the cell. First, each of the three potential DNA binding modules of σ is capable of only very weak interaction with their respective DNA elements. None of these individual interactions is sufficient for promoter complex formation. Second, in free sigma, the distance between DNA binding domains is inappropriate for simultaneous interaction with their respective promoter elements. Simultaneous interaction between DNA binding domains of σ and their target promoter elements only becomes possible upon holoenzyme formation. Third, one or more autoinhibitory interactions prevent promoter recognition by free sigma. The autoinhibition is relieved upon the holoenzyme formation.

At most bacterial promoters, σ^{70}-type RNAP holoenzymes recognize sequence elements centered ∼ 10 and 35 nucleotides upstream of the initiation point. Genetic, biochemical and structural data show that σ conserved region 4.2 recognizes the −35 consensus promoter element ([7] and references cited therein). At conditions of very high protein concentrations needed for crystallization, a proteolytic fragment of *Thermus aquaticus* sigma A containing region 4 specifically interacted with a double-stranded DNA fragment containing the −35 promoter consensus element [7]. In agreement with earlier predictions, the X-ray crystal structure of this complex revealed that region 4.2 assumes a helix-turn-helix conformation typical of many bacterial DNA-binding proteins and binds the −35 element through specific interactions in the major groove [7]. Sigma region 4.2 is required for promoter complex formation on most promoters, and several bacterial regulators affect efficiency of promoter initiation by interacting with region 4.2 and either strengthening (activators) or weakening (repressors) region 4.2 interactions with the 35 promoter element.

Genetic, biochemical and structural data show that σ conserved region 2.4 recognizes the –10 consensus promoter element ([14,19,23] and references cited therein). Isolated σ subunits or σ fragments containing region 2 recognize the double-stranded −10 promoter element very poorly if at all. However, σ subunits or σ fragments containing region 2 specifically bind the non-template strand of the −10 promoter consensus element in the presence of RNAP core [14,19].

The primary interaction between σ and RNAP core occurs through strong contacts between sigma regions 1.2 and 2.2 and evolutionarily conserved coiled-coil element of the β′ subunit (colored white in Fig. 1b, see Ref. [1,18,25]), a platform-like structure located at the floor of the DNA binding channel in the β′ jaw. A peptide corresponding to σ regions 2.1 and 2.2 inhibits the holoenzyme formation [24]. On the other hand, a peptide corresponding to β′ coiled-coil induces efficient recognition of single-stranded −10 promoter consensus element by σ^{70} or its fragments containing region 2 [28]. Evidently, interaction of σ with the β′ coiled-coil removes an autoinhibitory interaction that interferes with specific recognition of the −10 element DNA by free σ. The detailed nature of this interaction is unknown.

There exists a minor class of bacterial promoters which lack recognizable −35 promoter element and whose −10 elements are extended by an upstream dinucleotide motif 5′-TG-3′. Genetic and structural data show that specific interactions between σ region 2.5 and the TG motif account for efficient promoter complex formation on promoters of this class [3,19]. These additional contacts are strong enough to make promoter complex formation on some extended −10 promoters independent of σ region 4.2 and –35 promoter element interaction.

In the context of an RNAP holoenzyme, regions 2.4 and 4.2 of different sigmas recognize promoter elements with different sequences. As a result, RNAP holoenzymes formed with different sigma subunits are capable of recognizing different types of promoters. Global changes in gene expression during heat shock in Gram-negative bacteria, sporulation in Gram-positive bacteria and development of certain bacterial viruses are achieved by changing RNAP specificity through σ substitution. There is currently no data whether regions 2.5 from different sigmas can recognize different extended −10 motifs and thus contribute to the specificity of promoter recognition.

In order for template-directed RNA synthesis to occur, promoter DNA has to become locally melted (opened). In the catalytically competent open promoter complex the melting extends from ca. −12 to +3 positions and thus includes the entire −10 promoter element. While sigma clearly contributes to the stability of open promoter complex through interactions with the −10 element in its single-stranded form, the role of σ in the process of DNA melting is not clear. Region 2.3 has several conserved aromatic residues which may participate in the nucleation of melting. However, the propagation of melting towards the transcription initiation start site does not appear to involve σ and may be triggered by establishment of contacts between downstream DNA and RNAP core subunits [22].

Luminescence resonance energy transfer measurements show that in free σ^{70}, the interdomain distance between regions 2.4 and 4.2 is ca. 40 Å, which is much shorter than the distance between the −10 and the −35 promoter elements (assuming they are separated by 17 bp of B-form DNA). Upon formation of the holoenzyme, *E. coli* RNAP core induces a conformational change that brings σ regions 2 and 4 further apart and makes possible −10/−35 promoter complex formation by allowing simultaneous recognition of the −10 and −35 promoter elements [5]. The mechanism of this change has recently been elucidated; it involves protein–protein interactions between a flexible element of RNAP core enzyme, the β subunit flap domain, and σ region 4 [11]. In the core RNAP structure, the flap does not contact any other part of RNAP and is kept in place by crystal contacts [29]. In RNAP holoenzyme structures, the β flap interacts with sigma region 4, and its position relative to the main body of RNAP differs radically from its position in the core enzyme structure [18,25]. When the β flap is removed by mutation, RNAP holoenzyme can form, but the interdomain distance between σ regions 2 and 4 remains short. As a consequence, RNAP holoenzyme lacking the β flap is unable to recognize −10/−35 promoters. As expected, the β flap is not required for recognition of extended −10 promoters [11]. The results suggest that σ region 4 interaction with the β flap triggers the flap to move, thereby increasing the interdomain distance between σ regions 2 and 4 and allowing simultaneous recognition of the −10 and the −35 promoter consensus elements.

The length of the β flap and the extent of the β flap movement during the holoenzyme formation may be the underlying cause of strict requirement for the length (17 + / − 1 bp), but not the sequence, of the spacer DNA separating the two promoter consensus elements. Further, it is possible that factors that affect the β flap movement might permit recognition of promoters with suboptimal spacers. More generally, there may exist a class of regulatory factors that affect promoter recognition by either disrupting or stabilizing the interaction between the β flap and region 4 of σ. In fact, an antisigma protein that downregulates *H. pylori* σ^{28}-dependent transcription has been shown to disrupt σ^{28}–β flap interaction [8]. It is conceivable that some transcriptional regulators currently thought to target sigma region 4.2 may also influence the interaction of σ with the β flap.

The strength of σ region 4–β flap interaction could indirectly influence the specificity of promoter recognition. Consider the case of two *E. coli* σ factors, σ^{70} and σ^{S}. The σ^{70} and σ^{S} holoenzymes have different physiological functions. The σ^{70} holoenzyme is responsible for transcription of house-keeping genes; the σ^{S} holoenzyme is responsible for transcription of genes required for stationary phase survival. In the context of RNAP holoenzyme, σ^{70} and σ^{S} regions 2.4 and 4.2 have identical consensus promoter elements, TATAAT and TTGACA, respectively [10]. Despite having the same preferred promoter elements, the σ^{70} and σ^{S} holoenzymes exhibit different promoter specificities in vivo and in vitro. The σ^{S} holoenzyme appears to be more tolerant to deviations from the consensus in the −35 element and reaction conditions that weaken RNAP–promoter interactions such as increased salt concentrations [10]. The reasons for this behavior are unknown. A two-hybrid interaction screen revealed that σ^{S} interaction with the β flap is several orders of magnitude stronger than σ^{70}–β flap interaction [11]. It is therefore attractive to speculate that the stronger interaction with the β flap (and consequent change in the β flap orientation) firmly positions σ^{S} region 4 in the vicinity of the −35 promoter element and allows the establishment of favorable contacts between σ^{S} region 4.2 and suboptimal −35 promoter elements. Conversely, weak interaction of the β flap with σ^{70} region 4 makes specific region 4.2 contacts with the −35 promoter boxes essential for promoter complex formation.

Two remaining conserved regions of proteins of the σ^{70} family, regions 1.1 and 3, also contribute to the overall efficiency of promoter utilization by RNAP holoenzyme, but through entirely different mechanisms. Region 1.1 has long been suspected to be responsible for autoinhibition of promoter DNA recognition by free σ. Mutant σ^{70} lacking region 1.1, but not the wild type σ^{70} can recognize the −35 promoter consensus element. Biophysical analysis indicates, however, that region 1.1 does not interact with region 4 in free σ and the inhibition of DNA binding may thus be an indirect consequence of the highly negative charge of region 1.1 [6].

Region 1.1 is not seen in RNAP holoenzyme structures, but the point of its attachment to the rest of the σ subunit is located close to RNAP active site [18,25]. Biophysical data indicate that in RNAP holoenzyme, region 1.1 occludes the part of RNAP DNA binding channel where double-stranded DNA downstream of the transcription initiation start point is proposed to bind [15]. It has therefore been proposed that in the holoenzyme, this negatively charged region acts as a DNA mimic that must be displaced upon promoter complex formation. Indeed, biophysical data indicate that the position of region 1.1 is changed upon holoenzyme interaction with promoter DNA [15]. The relative strengths of the RNAP–downstream DNA interaction (which itself is affected by the strength of interaction with promoter consensus elements and efficiency of promoter melting) versus the RNAP–σ region 1.1 interaction were therefore proposed to modulate the efficiency of promoter complex formation [15]. According to this view, one would expect that removal of region 1.1 will make promoter complex formation more efficient. Instead, RNAP holoenzyme reconstituted with σ^{70} lacking region 1.1 is strongly deficient in later steps of promoter complex formation on most promoters and takes a much longer time to form stable promoter complexes [27]. Thus, further analysis is needed to elucidate the exact function of this interesting domain of σ.

Most bacterial transcripts are initiated with a purine nucleoside triphosphate. σ^{70} region 3.2 can be crosslinked to initiating purine substrate analogue with a crosslinkable group positioned at the γ-phosphate [21]. The result implies that σ may participate in the formation of the initiating site of the enzyme. Indeed, in RNAP holoenzyme structures, region 3.2, which is part of the linker connecting σ ND2 and CD, makes a deep excursion towards the RNAP active center (indicated by catalytic Mg^{2+} ions in Fig. 1b) and in so doing occludes much of the RNA exit pathway [18,25]. This position of σ region 3.2 may provide a structural explanation for the phenomenon of abortive transcription initiation. Abortive transcription is a universal feature of all DNA-dependent RNA polymerases and can be thought of as a price that enzymes of this class have to pay for being able to initiate template-dependent synthesis in the absence of a primer. Multiple short abortive transcripts are synthesized by promoter-bound RNAP before the enzyme produces a transcript that is long enough to allow the formation of stable ternary (RNAP-RNA-DNA) transcription elongation complex and promoter escape. The location of σ region 3.2 in the holoenzyme structure makes a clash with the growing RNA chain inevitable and this clashing may cause short transcripts to be expelled from the complex. Indeed, analysis of transcription by RNAP holoenzyme reconstituted from σ that lacked region 3 showed that few abortive products were produced compared to transcription by the wild type enzyme [7]. Eukaryotic RNAPs are also known to perform abortive initiation prior to escape into transcription elongation ([9] and references therein). This implies that a structural feature analogous to σ region 3.2 is provided by one of eukaryal general transcription initiation factors or that abortive initiation is not solely due to a clash between region 3.2 and the growing RNA.

Since region 3.2 stands in the way of the growing nascent RNA chain, it must be displaced once RNA reaches a length of 8–10 nucleotides, a critical point at which a stable ternary elongation complex is formed on most promoters and σ is thought to leave the complex. Further, σ region 4.2–β flap contacts also have to be broken, since the β flap is thought to interact with secondary structure elements in the nascent RNA during elongation [26]. However, there appears to be no structural requirement for the primary σ-core interaction, that between σ region 2.2 and β′ coiled-coil element, to be broken upon escape to elongation. The first evidence that stable ternary elongation complexes may contain σ came from the analysis of regulatory pause in transcription elongation induced by bacteriophage λ Q protein. It has been shown that when RNAP transcribes through a promoter-proximal DNA that contains a sequence corresponding to the extended −10 consensus element, σ^{70} does not dissociate from the complex, but instead "hops" and establishes specific contacts with the non-template strand of the extended −10 consensus element through which RNAP had transcribed [20]. The σ-DNA interactions slow transcription elongation, giving the λ Q protein enough time to modify the elongation complex and convert it into a form that is unable to recognize terminators [20]. Recent reports claim that σ may remain bound to the transcription elongation complex in vitro even in the absence of specific recognition sequences in transcribed DNA [2,17]. The physiological significance, if any, of this phenomenon is not clear yet.

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