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Structural analysis of DNA wrapping in bacterial transcription initiation complex by  
transmission electron microscopy and single particle analysis

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## ABSTRACT

FLOREZ ARIZA, A. **Structural analysis of DNA wrapping in bacterial transcription initiation complex by transmission electron microscopy and single particle analysis.** 2018. 93p. Dissertação (Mestrado em Ciências) - Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, 2018.

The transcription initiation is the first step in gene expression and an important regulation step in all living organisms. In bacteria, it has been proposed that DNA bending and its wrapping on the surface of *E. coli* RNAP might facilitate the opening of the transcription bubble, which is necessary for the initiation of gene transcription. In this work, it is shown the first structural study to evaluate a DNA wrapping model, including its length and the relative position in the bacterial transcription initiation complex (RP complex), assembled between RNA polymerase- $\sigma^{70}$  holoenzyme (RNAP) and a  $\lambda$ PR promoter (-100 to +30 wild type). RP complex was prepared and negatively stained with 2% uranyl acetate on a thin-carbon coated grid and the data acquisition of 500 images was performed in a JEM-2100 (JEOL, Japan) microscope equipped with an F-416 CMOS camera (TVIPS, Germany). Single particle analysis of 16,015 particles, grouped in 666 class-averages, was conducted using IMAGIC 4D software (Image Science, Germany) to obtain a three-dimensional model of the RP complex at 20Å resolution. After the rigid-body fitting of the RNAP crystallographic structure (PDB 4YG2) and the modeled DNA promoter, it was observed that the regions 1.2 and 4.2 of the  $\sigma^{70}$  subunit interacts with the consensus zones, -10 and -35 hexamers of the promoter. Furthermore, it was possible to observe that  $\alpha$ CTDs (C-terminal domain) in both alpha subunits would be oriented to facilitate the interaction with the first and second UP-elements regions, respectively (centered around -50 and -75 positions in the promoter). These was enabled by the presence of the characteristics motifs helix-hairpin-helix in these domains. In addition, the downstream DNA, from the transcription bubble, appears to be inside the protein main channel, oriented in a way to enable interactions with the RNAP clamp and jaws. Finally, it was observed that the DNA wrapping has ~32 nm of total length and involves a promoter bent of ~255° around the RNAP surface. The 3D-model obtained in this study is the very first direct structural confirmation of the DNA promoter wrapping in a bacterial transcription initiation complex.

**Keywords: Transcription initiation Complex. RNA polymerase. DNA wrapping. Transmission electron Microscopy. Single particle analysis.**



## RESUMO

FLOREZ ARIZA, A. **Análise estrutural do enovelamento do DNA no complexo da iniciação de transcrição bacteriano usando microscopia eletrônica de transmissão e análise de partículas isoladas.** 2018. 93p. Dissertação (Mestrado em Ciências) - Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, 2018.

A iniciação da transcrição é o primeiro passo na expressão gênica e importante ponto de regulação em todos os organismos vivos. Em bactérias, foi proposto que o enovelamento do DNA na superfície da RNAP de *E. coli* pode facilitar a abertura da bolha de transcrição, necessária para o início da transcrição gênica. Neste trabalho, é apresentado o primeiro estudo estrutural direto para avaliar o comprimento do enovelamento do DNA e sua posição no complexo de iniciação da transcrição bacteriana (complexo RP), montado entre a holoenzima RNA polimerase- $\sigma^{70}$  (RNAP) e um promotor  $\lambda$ PR (-100 para +30, tipo selvagem). Amostras do complexo RP foram preparadas e contrastadas negativamente com 2% de acetato de uranila em uma grade com filme fino de carbono e a aquisição de 500 imagens foi realizada em um microscópio JEM-2100 (Jeol, Japão) equipado com uma câmera CMOS F-416 (TVIPS, Alemanha). A análise de partículas isoladas de 16.015 partículas, agrupadas em 666 médias de classe, foi conduzida usando o software IMAGIC 4D (Image Science, Alemanha) para obter um modelo tridimensional do complexo RP, a 20Å de resolução, estimado pelo critério de  $\frac{1}{2}$  bit. Após o ajuste de corpo rígido da estrutura cristalográfica da RNAP (PDB 4YG2) e do promotor de DNA modelado, observou-se que as regiões 1.2 e 4.2 da subunidade  $\sigma^{70}$  interagem com as zonas de consenso, hexâmeros -10 e -35, do promotor. Além disso, foi possível observar que os  $\alpha$ CTDs (domínio C-terminal) em ambas as subunidades alfa estariam orientados para facilitar uma possível interação com a primeira e segundas regiões dos elementos UP, respectivamente (centradas em torno das posições -50 e -75 do promotor). Estas seriam possíveis devido à presença de alguns motivos de características hélice-grampo-hélice nesses domínios. Além disso, a região do promotor, *downstream* da bolha de transcrição, parece estar dentro do canal principal da proteína, orientado de forma a possibilitar interações com o *clamp* e *jaw* da RNAP. Finalmente, foi observado que o comprimento total do enovelamento de DNA envolve cerca de 32 nm e 255° de rotação do DNA ao redor da superfície da RNAP. Portanto, este modelo 3D é a primeira confirmação estrutural direta do enovelamento de DNA em um complexo bacteriano de iniciação da transcrição.

Palavras-chave: Complexo da iniciação da transcrição. RNA polimerase. Enovelamento do DNA. Microscopia eletrônica de transmissão. Análise de partículas isoladas.





## 1 INTRODUCTION

### 1.1 Bacterial transcription initiation complex

#### 1.1.1 General aspects

The assembly of transcription initiation complex (RP) is the first step for the gene expression and an important regulation point for all living organisms. One of the major players in the formation of this complex is the RNA polymerase holoenzyme (RNAP), which is formed from the binding of the core enzyme with the sigma factor. Bacterial core RNA polymerase consists of five subunits,  $\alpha_2\beta\beta'\omega$ , with a molecular mass of 370 kDa (Figure 1).<sup>1</sup> This core enzyme is capable of non-specific DNA binding and starts the RNA synthesis from DNA ends or nicks. The transcription machinery must recognize with high specificity the sites where genes begin, called promoters, among a huge extension of non-specific binding sites offered by genomic DNA. The core enzyme needs to bind the sigma factor ( $\sigma$ ) to form the transcriptionally active holoenzyme. This  $\sigma$  factor has the capability to specificity recognize some consensus regions on the DNA promoter and thus start the process of forming a stable RP complex.<sup>2,3</sup> The nature of the sigma factor with which the core enzyme would interact depends on the environmental conditions and the identity of the promoter. Bacteria possess an essential housekeeping  $\sigma$  factor that controls many different promoters. In *Escherichia coli* it is the  $\sigma^{70}$  (molecular weight of 70 KDa) and in *Bacillus subtilis* and other Gram-positive bacteria it is the  $\sigma^A$ .<sup>4</sup> In the case of the  $\sigma^{70}$  factor, it recognizes the -35 and -10 hexamers consensus zones, upstream from the transcriptional start site (+1) of the promoter (Figure 1).<sup>5</sup> The sigma factor binding to the consensus zone results in the formation of the closed complex (RPC). That induces significant conformational changes in both, the RNAP and the DNA, including the transcription bubble formation, to form a stable transcription initiation open complex (RPo).<sup>2,5</sup>

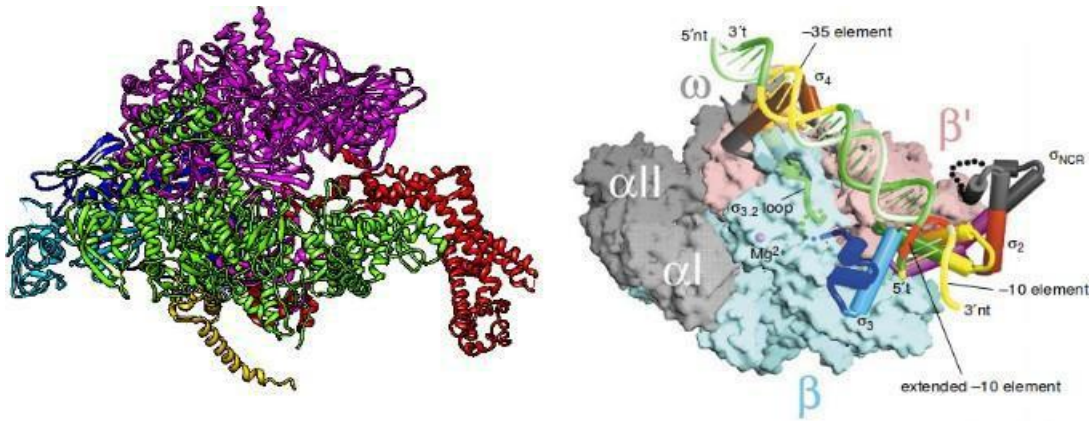


Figure 1 - Crystallographic structure of *E. coli* RNA polymerase sigma 70 holoenzyme (left: PDB 4YG2). The  $\sigma^{70}$  is in red,  $\omega$  in yellow,  $\beta$  in magenta,  $\beta'$  in green, and the alpha subunits are shown as follows:  $\alpha^I$  in blue and  $\alpha^{II}$  in light-blue. Structure of the close RP complex between the RNAP holoenzyme and fork-junction DNA. The core enzyme is shown in surface representation and the sigma factor is shown as a C $\alpha$  backbone worm. It can be observed that the -35 and the -10 elements (in yellow) are recognized by the region 4 and 2 of the sigma subunit respectively.

Source: Adapted from MURAKAMI;<sup>6</sup> MURAKAMI; DARST.<sup>2</sup>

All these conformational changes are associated with kinetic intermediates which vary in stability and lifetime among different promoters, suggesting that the DNA sequences determine different configurations of the RP complex.<sup>7</sup>

### 1.1.2 The $\sigma^{70}$ factor

The sigma factor plays a crucial role in the specific promoter recognition. The  $\sigma^{70}$  subunit has four different domains or regions (from 1 to 4) and one non-conserved region (NCR). Each of these regions has different sub-domains (Figure 2).

There are several biochemical and structural studies supporting the idea that the DNA recognition and binding, is accomplished by the interaction of the  $\sigma_{4.2}$  sub-domain with the -35 hexamer, while  $\sigma_{3.0}$  sub-domain binds to the -10 hexamer. The  $\sigma_{1.2}$  and  $\sigma_{2.1} - \sigma_{2.4}$  regions interact with the transcription bubble<sup>8</sup> and the  $\sigma_{NCR}$  interacts with the promoter DNA upstream of the -10 element.<sup>9</sup>

The TGn element on the promoter, a consensus region which has been reported to be crucial for an optimal activity for different promoters is recognized by the  $\sigma_{3.0}$  subdomain, possibly through E458 and H455 residues as was shown by some mutagenesis assays.<sup>10</sup> This interaction was also shown to be present in *T. aquaticus* RNAP holoenzyme-fork DNA complex, through analogous residues in the  $\sigma^A$  factor.<sup>11</sup>

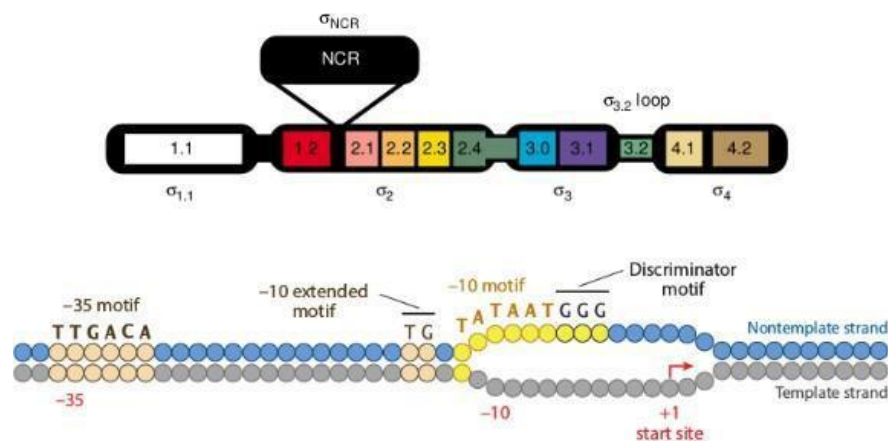


Figure 2 - (A) Sketch of the *E. coli*  $\sigma^{70}$  sub-domains. (B) Schematic representation of the DNA canonical promoter regions recognized by the  $\sigma^{70}$  sub-domains to form the RP complex. Source: Adapted from FEKLÍSTOV;<sup>5</sup> MURAKAMI; DARST.<sup>2</sup>

Furthermore, the  $\sigma_{1.1}$  sub-domain seems to perform a regulatory role. Studies using NMR spectroscopy showed that in absence of RNA core enzyme, the  $\sigma^{70}$  is auto-inhibited to DNA binding. It is suggested that there is an "intra molecular interaction" between the 4.2 sub-domains, which should bind to -35 hexamer, with the region  $\sigma_{1.1}$ .<sup>12</sup> Moreover, there is the hypothesis that when the  $\sigma^{70}$  binds to the DNA, the  $\sigma_{1.1}$  blocks the "primary" or main channel of the enzyme, during the formation of the transcription initiation complex. After major conformational changes, the  $\sigma_{1.1}$  is expelled, allowing the DNA promoter to completely enter the primary channel (Figure 3). This hypothesis is supported by the structural study of the Phage T7 Gp2 protein inhibition of the RNAP- $\sigma^{70}$ , which shows that there is a ternary interaction between the  $\sigma^{1.1}$ -Gp2- $\beta$ 'jaw, that prevent the  $\sigma^{1.1}$  to leave the main channel inhibiting the function of the enzyme.<sup>13</sup>

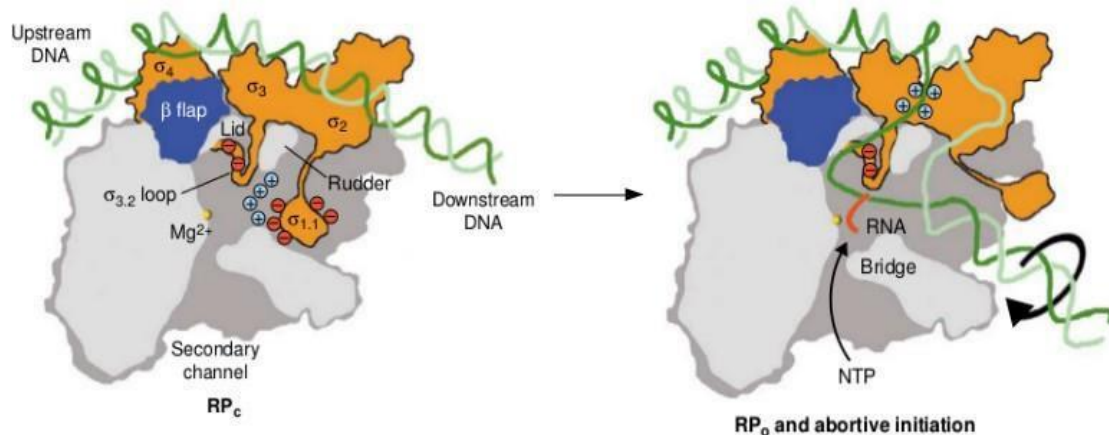


Figure 3 - (A) Cartoon representation of conformational changes in the transition from RP<sub>c</sub> to the RP<sub>o</sub> complex. In the figure the core *E. coli* RNA polymerase is shown in grey – except the β-flap shown in blue- and the σ<sup>70</sup> factor is in orange. Each σ domain is indicated by name. Once the RP<sub>o</sub> complex is formed, the σ<sub>1.1</sub> is out of the main channel.

Source: Adapted from MURAKAMI; DARST. <sup>2</sup>

Some of the conformational changes, occurred during the formation of the transcription initiation complex, involve portions of DNA that are considerably distant to the transcription-starting site. These regions on the promoter, known as UP-elements, are upstream regions to the -35 hexamer, and generally possess phased A-T tracks with a certain length that could interact with the α subunits of RNAP.

### 1.1.3 Alpha subunits and UP-elements

The RNAP has two alpha subunits both have the same polypeptide chain. Each α subunit has two independent folded domains: the amino-terminal domain (αNTD, residues 8–233), responsible for dimerization and interactions with β and β', and the carboxyl-terminal domain (αCTD, residues 245–329). A flexible tether, known as α-linker, of about 18 residues length, connects the two domains.<sup>14</sup>

There is experimental evidence that both αCTDs have a key role in the formation of transcription initiation complex. These are capable of binding DNA specific sequences, commonly called UP-elements. These UP-elements, located upstream the -35 hexamer, are not conserved as high as the -10 and -35 hexamers, but exhibit interesting features, as to own A-T rich regions (of ~10 bp length or more,

separated with a periodicity of  $\sim 10.3$  bp), providing a particular curvature to the DNA, which seems to be related with the promoter strength and transcription regulation.<sup>15,16</sup>

In this sense, biochemical and genetic screening studies showed that the  $\alpha$ CTDs can bind UP-elements mainly through seven residues: L262, R265, N268, C269, G296, K298, S299, all of which belong to a helix-harping-helix motif present in this domain.<sup>17</sup> Even more, X-ray crystallography studies showed that  $\alpha$ CTD is oriented to interact with the DNA minor-groove and backbone of the UP-elements. Moreover, because these UP- elements can be in close proximity to the -35 hexamers, the  $\alpha$ CTD could be possibly interacting with the region 4 of the  $\sigma$  factor, aiding to stabilize the RP complex.<sup>18,19</sup>

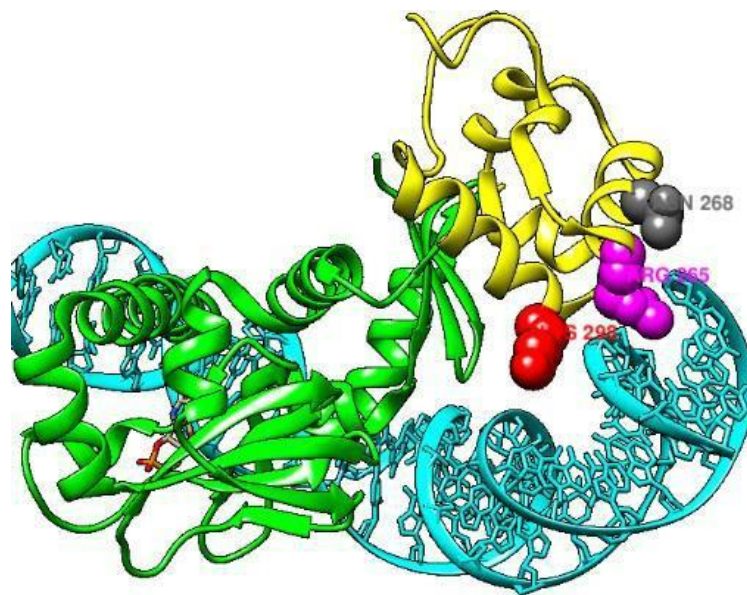


Figure 4 – Crystallographic structure of the ternary complex CAP-DNA- $\alpha$ CTD. The CAP protein is in green, the  $\alpha$ CTD in yellow and the DNA UP-element region is in cyan. In the figure can be observed that the residues (in spheres) lys-298 (red), arg-265 (magenta) and asn-268 (grey), are oriented to interact with the minor groove and backbone of the DNA UP-element.

Source: Adapted from BENOFF.<sup>18</sup>

The effects of the UP-elements on promoter strength were established by different studies that evaluated how different upstream sequences can affect the relative activity among promoters. In this sense, Ross and co-workers showed that the two UP- elements (proximal UP-element, -38 to -41 and distal UP-element, -51 to -60) of the wild-type *rrnB* P1 promoter, improved the *in vivo* transcription activity of the *lacZ* core promoter (+52 to -37) up to ~33 fold when having both UP-elements.<sup>20</sup> But even each one of the UP- elements, separately, were able to improve the transcription activity. Moreover, it was reported in the same study that *E. coli* RNAP holoenzyme lacking  $\alpha$ CTDs was not able to interact with the UP-element regions (confirmed by foot-printing assays), and that the transcription activity was completely dependent on this interaction.<sup>20</sup> Furthermore, this kind of UP-elements/ $\alpha$ CTDs interactions seems to be present in different bacterial phyla. For example, a study performed in *Helicobacter pylori*, by Nuclear Magnetic Resonance (NMR), showed that both  $\alpha$ CTDs interact directly with the UP-element of the DNA promoter and NikR protein to form a stable ternary transcription initiation complex.<sup>21</sup>

The interactions described above, involve DNA regions centered on -45 to -60 bp upstream the transcription start site. However, for some promoters, like the  $\lambda$  PR promoter, it was reported that the RNAP- $\alpha$ CTDs was able to interact with far upstream zones of the DNA, up to -90 bp upstream. This kind of new interaction between those UP- elements and RNAP would only be possible if the DNA promoter underwent a significant curvature around the RNAP surface. This interesting and relatively new phenomenon, known as *DNA wrapping*, will be detailed in the following section.

#### 1.1.4 The "wrapping" phenomenon

As mentioned before, the interaction between those upstream promoter regions and RNAP would only be possible if the DNA promoter underwent a significant curvature around the RNAP surface, hinting a DNA wrapping formation. In this sense, experimental evidence suggest that DNA wrapping would entail a DNA curvature of  $\sim 300^\circ$  around the RNAP surface (Figure 5).<sup>22</sup> Concerning the  $\lambda$ PR promoter, different studies by Atomic Force Microscopy (AFM), proposed that in the RP complex, the promoter was forming a "wrapping" of  $\sim 30$  nm ( $\sim 90$  bp)

around the RNAP surface (Figure 6). Also, it was reported that this DNA wrapping is reduced to  $\sim 3$  nm when the complexes are formed with RNAP lacking the  $\alpha$ CTDs, and to  $\sim 15$  nm when the  $\alpha$ -linker is missed. Those observations seemed to be according with different DNase and OH radical foot-printing assays, performed in previous studies.<sup>23–26</sup>

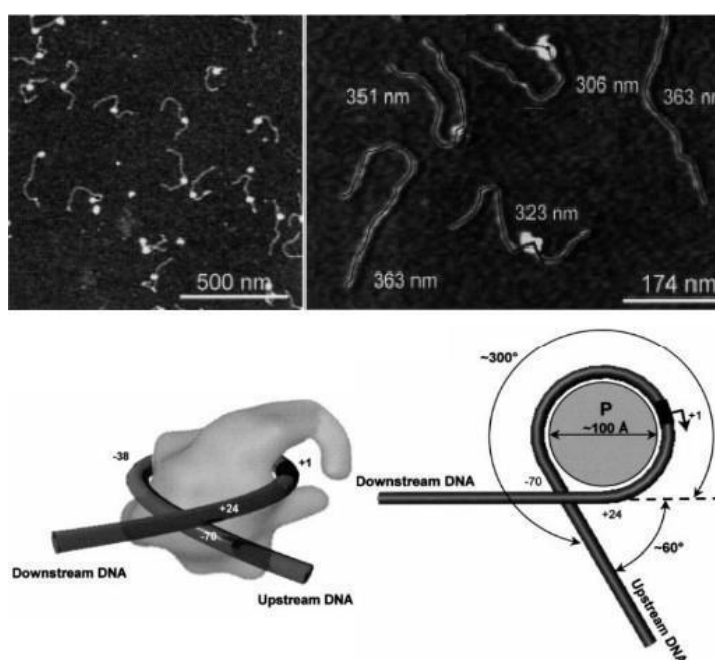


Figure 5 - A square patch of one AFM micrograph of RP complexes (top left) assembled between *E. coli* RNAP and a DNA fragment containing the  $\lambda$  PR wild type promoter (-100 to +34). A zoom in image (top right) showing the RP complexes and the measures of the DNA contour length free and in complex. Schematic representation of the RP complex (bottom), showing the RNAP in light grey and the DNA promoter in dark grey. The DNA trajectory has proposed around the RNAP surface, with a bend angle of  $300^\circ$ .

Source: Adapted from RIVETTI.<sup>22</sup>

Recently, a study using fluorescence resonance energy transfer (FRET), on the same system, measured the distance between fluorescent labels in positions +14 and -100 relatives to the transcription starting site (+1), confirming that these locations come close to each other in a way that is consistent with the proposed DNA wrapping.<sup>27</sup>

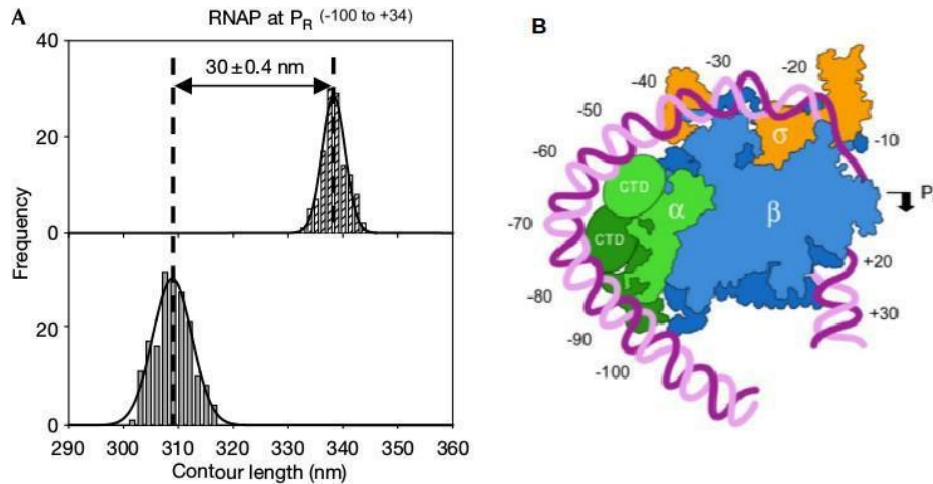


Figure 6 – (A) Contour length distributions of the DNA both in absence (dashed bars, top) and presence of the RNAP (gray bars, bottom). The DNA compaction in the RP complex is around 30 nm. (B) Cartoon representation of the RP complex, showing that UP-elements, centered around -50 and -90 bp, would be interacting with the  $\alpha$ CTDs

Source: Adapted from MANGIAROTTI.<sup>23</sup>

Furthermore, another study showed that the DNA wrapping length is significantly reduced by allosteric regulators like the guanosine tetra phosphate (ppGpp) and Dksa protein.<sup>28</sup> The wrapping phenomenon have been also suggested to be present in other promoters like *rrnB* P1, *hdeAB* and *tRNA<sup>tyr</sup>*;<sup>29–31</sup> and also for eukaryotes, as was observed for the adenovirus promoter in complex with the RNA pol II of *S. cerevisiae*.<sup>32,33</sup> All of these studies, seem to indicate that the DNA wrapping is a conserved phenomenon along different kind of promoters and directly related to the promoter strength and stability of the transcription initiation complex.

However, even with all the studies mentioned above, there is no direct structural evidence of the DNA wrapping. Until now, there are questions that remains without answer like which could be the protein domains possibly interacting with all the UP-elements? How could this be related to the formation of a stable RP complex? Which would be the real extension of the DNA wrapping, and the promoter regions involved? In the present work, we present the first direct structural study of DNA wrapping in the RP complex assembled between the *E. coli* RNAP holoenzyme (RNAP- $\sigma^{70}$ ) and the  $\lambda$  PR wild- type promoter (-100 to +30 bp). This study was carried out by transmission electron microscopy (TEM) and single particle analysis (SPA).



## 2 CONCLUSIONS AND FUTURE PERSPECTIVES

In this work, we successfully assembled the transcription initiation complex between the  $\lambda$  PR wild-type promoter (-100 to +30) and the *E. coli* RNA polymerase- $\sigma$ 70 holoenzyme (RP complex). Using Transmission Electron Microscopy and Single Particle Analysis, it was possible to determine the 3D-structure for this complex at 20 Å resolution. This 3D-model is the very first direct structural confirmation of the DNA promoter wrapping in a bacterial transcription initiation complex.

For this study, the RP complex preparation was performed according to previous biochemical and structural studies, primarily obtaining the RP open complex as the final product. Thus, even being the first direct structural confirmation of the wrapping existence, the suggested model only represents a conformation, the final stage, among all the possible ones, from the closed complex formation to the stable wrapped RP open complex.

The rigid-body fitting of RNA polymerase and the modeled DNA promoter into the 3D-map allowed proposing possible DNA-RNAP interactions that would enable the wrapping formation. According to this model, the  $\alpha$ CTDs, probably due to the flexibility of the  $\alpha$ -linker, would be the domains independently interacting with the UP-elements of the promoter. Specifically, the  $\alpha$ CTD<sup>I</sup> would be oriented to interact with the first UP element of the promoter (-45 to -58) and the  $\alpha$ CTD<sup>II</sup> would be oriented to interact with the second UP-element (-70 to -79). It was not possible to observe a clear density assigned to the third UP-element of the promoter (-90 to -100), which could suggest that this region is not interacting with the RNAP, during the final stage of wrapping formation. All these observations are strongly supported by different biochemical studies and other indirect structural analysis performed by independent techniques.

According to the proposed structural model, the promoter spans an interaction region with the enzyme from -79 to +18. Moreover, it was observed that the promoter wrapping implies an overall bending of  $\sim 255^\circ$ , as well as a total length of  $\sim 32$  nm, considering the distances between the ends, before and after the wrapping formation. Those observations have shown to be fairly in agreement with previous independent studies performed by optical tweezers, AFM and FRET.

As a future perspective, we expect that changing the incubation time and letting the RP complex to be assembled at lower temperatures would allow different transient states to be isolated. Thus, using Single Particles Cryo-EM, it would be possible to elucidate a “structural mechanism”, at atomic level, of the DNA wrapping during the transcription initiation. It would be also important to evaluate this biological system using mutant promoters and mutant proteins, and therefore getting new insights about the implication of promoter wrapping in the RP complex formation. Finally, other promoters could be tested for a structural study, to observe how the DNA-protein contacts would change according to the promoter nature, regarding different spacer or discriminator lengths as well as distinct UP-elements configuration.

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