Bacterial RNA polymerases: the wholo story

Katsuhiko S Murakami and Seth A Darst*

Recent structural and biophysical results have provided unprecedented insights into the structure and function of the bacterial RNA polymerase holoenzyme as it goes through the steps of transcription initiation. Comparisons with structural analyses of evolutionarily unrelated RNA polymerases reveal unexpected general features of the initiation process.

Addresses
The Rockefeller University, Box 224, 1230 York Avenue, New York, NY 10021, USA
*e-mail: darst@rockefeller.edu

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Abbreviations

RNAP	RNA polymerase
RP_c	closed RNAP–promoter complex
RP_o	open RNAP–promoter complex
Taq	*Thermus aquaticus*
TEC	ternary elongation complex
Tth	*Thermus thermophilus*

Introduction

RNA in all cellular organisms is synthesized by a complex molecular machine — the DNA-dependent RNA polymerase (RNAP). The catalytically competent core RNAP (subunit composition $\alpha_2\beta\beta'\omega$ with a molecular mass of about 400 kDa) is evolutionarily conserved in sequence, structure and function from bacteria to man [1–6]. Promoter-specific initiation of transcription requires an additional subunit, σ, which binds the core RNAP to form the holoenzyme (reviewed in [7]).

The past few years has seen an explosion of structural information on cellular RNAPs (reviewed in [8,9]). X-ray crystallographic structures of the bacterial core RNAP from *Thermus aquaticus* (Taq) [6,10], eukaryotic RNAP II from the yeast *Saccharomyces cerevisiae* [2] and a yeast RNAP II ternary elongation complex (TEC) [11] have been solved. These structures have provided insight into the elongating form of the cellular RNAPs with a level of detail previously unimagined.

As we review here, this past year has seen a new wave of structural [12•,13••–15••] and biophysical [16•] results that shed unprecedented light on the steps of bacterial transcription initiation, with the σ factor in the spotlight. Comparisons of these data with structural analyses of evolutionarily unrelated RNAPs uncover surprising general features of the transcription initiation process.

Bacterial RNAP holoenzyme

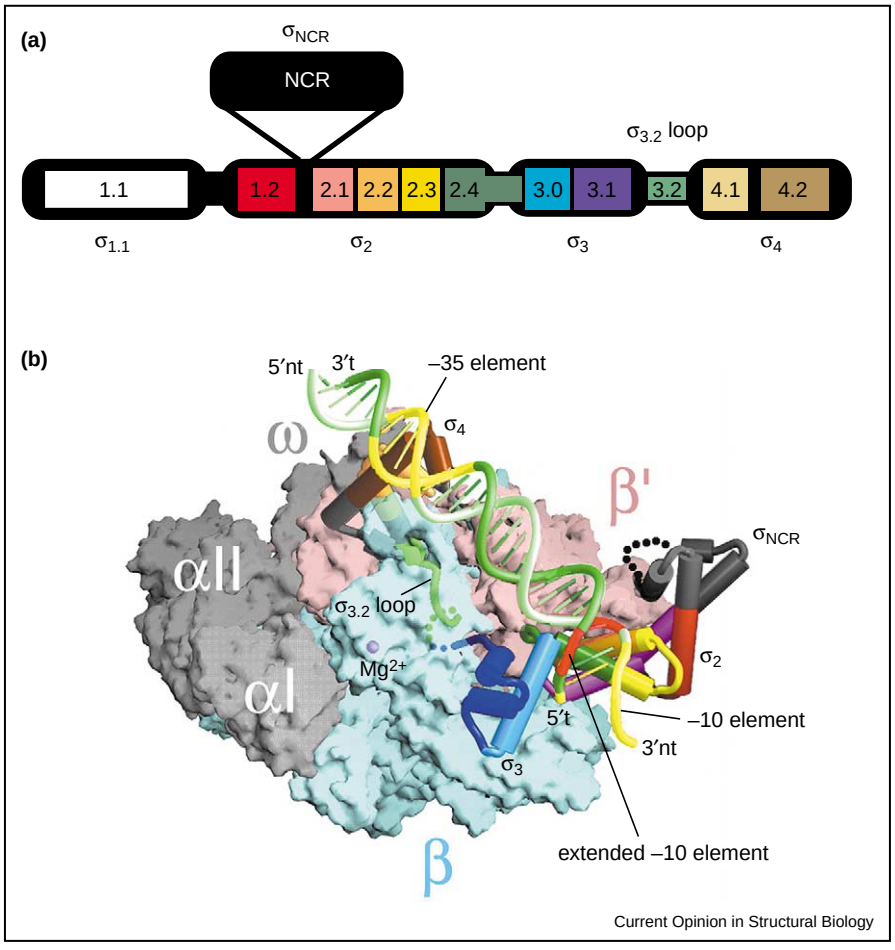
The X-ray structure of Taq core RNAP revealed a molecule shaped like a crab claw, with an internal channel of 27 Å in diameter. The molecule is about 150 Å long (from the back to the tip of the claws), 115 Å tall and 110 Å wide. The enzyme active site is located on the back wall of the channel, where an essential Mg^{2+} ion is bound. The overall shape and size is similar to yeast RNAP II, and the folds of highly conserved segments around the active site of these two enzymes are essentially identical [2,3,8,9].

Most bacterial σ factors belong to a homologous family that is closely related to σ^{70} from *Escherichia coli*, with distinct regions of highly conserved sequence [17,18] (Figure 1a). Group 1 (or primary) σ factors direct most transcription during log-phase growth. Structural analysis shows that group 1 σ factors comprise four, flexibly linked domains, $\sigma_{1.1}$, σ_2, σ_3 and σ_4, that contain conserved regions 1.1, 1.2–2.4, 3.0–3.1 and 4.1–4.2, respectively ([12•]; and A Shekhtman *et al.*, unpublished data). A nonconserved region (σ_{NCR}) inserted between regions 1.2 and 2.1 is not related in size, sequence or structure (Figure 1a).

In the holoenzyme, the globular domains of σ are spread out across the upstream face of the RNAP crab claw [14••,15••] (Figure 1b). The promoter-binding determinants of σ, σ_2 (−10 element) and σ_4 (−35 element) are solvent exposed and spaced according to their cognate DNA elements. The σ_3 and σ_4 domains are separated by 45 Å in the holoenzyme. This distance is spanned by an extended 33-residue linker, comprising primarily σ region 3.2 (the $\sigma_{3.2}$ loop), which loops into the RNAP active-site channel and then winds its way out through the RNA exit channel.

The interaction between σ and the RNAP core is very stable, with a dissociation constant estimated to be about 10^{-9} M [19]. Each of the σ domains, as well as the linkers that connect them, make interactions with the core RNAP. Nevertheless, when RNAP enters the elongation phase of transcription, the σ factor is generally released. These seemingly contradictory properties are explained by the architecture of the σ factor. The simultaneous, independent binding of discrete structural elements of σ (the $\sigma_{1.1}$, σ_2, σ_3 and σ_4 domains and the $\sigma_{3.2}$ loop) to

Figure 1



Structure of bacterial σ factor and the RNAP holoenzyme. **(a)** Sequence architecture of group 1 σ factors. The black bar represents the primary sequence of σ. The conserved regions [17,18] are labeled and color coded. The domain architecture of σ is indicated (structured domain, thick bar; unstructured flexible linker, thin bar), with the structured domains labeled beneath. **(b)** Structure of the complex between the RNAP holoenzyme and fork-junction DNA [14••]. The core component of the holoenzyme is shown as a molecular surface, with the αI, αII and ω subunits shown in gray, the β subunit in cyan, and the β′ subunit in pink. The σ factor is shown as a Cα backbone worm with its α helices shown as cylinders, colored according to the conserved regions shown in (a). The DNA phosphate backbone is shown as a worm, with the template strand (t) in dark green and the nontemplate strand (nt) in light green, except for the −35 and the −10 elements, which are yellow, and the extended −10 element, which is red. Figure 1b was generated using grasp [57].

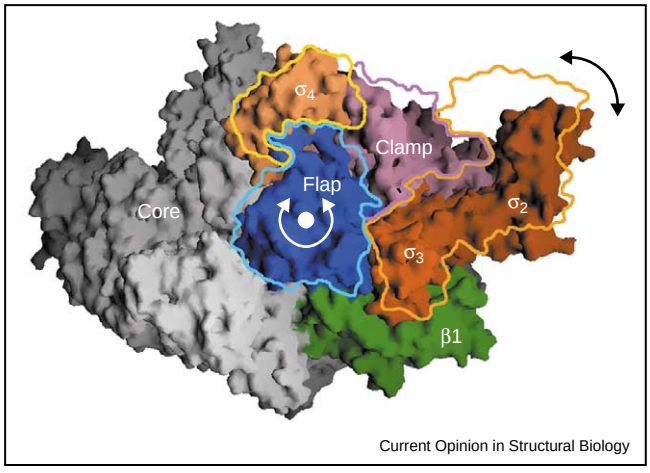
different parts of the core provide high-affinity binding without any one interaction between the core and an individual σ domain being particularly stable. Stepwise structural transitions during initiation could induce the dissociation of individual σ domains, one by one, effecting the eventual release of σ (see below).

Bacterial RNAP holoenzyme bound to promoter DNA

Two crystal structures shed light on the binding of the holoenzyme to promoter DNA. First, the complex of isolated σ_4 with −35 element DNA was solved at 2.4 Å resolution, providing details of the protein–DNA interactions and of a 36° bend in the DNA around the helix–turn–helix DNA-binding motif of σ_4 [12•]. Second, to visualize promoter binding in the context of the whole holoenzyme, Taq holoenzyme was crystallized with fork-junction promoter DNA [14••] containing double-stranded DNA from the −12 position to upstream regions (including the −35 element), only single-stranded, nontemplate DNA from the −11 to the −7 position (the −10 element), and no downstream DNA [20].

The RNAP holoenzyme binds sequence-specifically to fork-junction DNA, and the complex mimics many properties of the normal open complex [20,21]. The promoter DNA lies across one face of the holoenzyme, completely outside the RNAP active-site channel (Figure 1b). All sequence-specific contacts with the conserved promoter elements are mediated by the σ factor.

Figure 2



Mobile modules and conformational flexibility of the RNAP holoenzyme. The Taq RNAP holoenzyme [13••] is shown as a gray molecular surface, with the mobile modules color coded as follows: the relatively immobile 'core' domain is gray, the β1 subunit is green, the β flap is blue, the clamp is pink, the σ_2–σ_3 domain is orange, and the σ_4 domain is brown (β2 is hidden behind β1 and not visible in this orientation). The positions of the σ_2–σ_3-clamp and the σ_4-flap in the Tth holoenzyme structure [15••] are shown as outlines. Generated using grasp [57].

Holoenzyme flexibility

On the basis of comparisons of published structures, the RNAP enzyme can be described as a 'core' module (containing the two α subunit N-terminal domains, ω and regions of β and β′ surrounding the active site) connected to four mobile modules (β1, β2, the β flap and the clamp) that frame the active-site channel and can move as rigid bodies with respect to the core module [13••] (Figure 2). Observed conformational changes are dominated by swinging motions of the clamp that open or close the main channel by more than 20 Å [2,11,13••, 14••,22,23]. The open state of the clamp is presumably important during initiation, when DNA must enter the active-site channel. Closure of the clamp may be coupled to the presence of the RNA–DNA hybrid during elongation [2,11] and is presumed to give rise to the remarkable processivity of transcription.

A comparison of holoenzyme structures (Figure 2) in which the channel is relatively closed (Taq holoenzyme) [13••,14••] or open (*Thermus thermophilus* [Tth] holoenzyme) [15••] shows that the σ_2 and σ_3 domains move together as a rigid body with the clamp, with σ_3 'sliding' past β1. The β flap and σ_4 make another rigid mobile module (σ_4-flap) in the holoenzyme. The σ_4-flap and the σ_2–σ_3-clamp move independently, modulating the distance separating σ_2 and σ_4, and the −10 and −35 recognition elements. This plasticity is likely to be important for recognizing promoters containing variably spaced −10 and −35 elements.

Function of the σ factor

The new structures invite hypotheses of how the different structural elements of σ realize the transcription initiation functions, as described below.

The $\sigma_{1.1}$ domain

The group 1 σ factors have a unique N-terminal extension of roughly 90 amino acids called region 1.1. This region is poorly conserved in sequence, although the characteristic acidity is preserved. For example, in Taq σ^A, fully a third of the residues are negatively charged. Two functions have been ascribed to $\sigma_{1.1}$. First, $\sigma_{1.1}$ autoinhibits promoter recognition by free σ factor [24,25]. This autoinhibition is relieved when σ binds core RNAP to form the holoenzyme. Second, $\sigma_{1.1}$ accelerates the formation of open complex at some promoters [26].

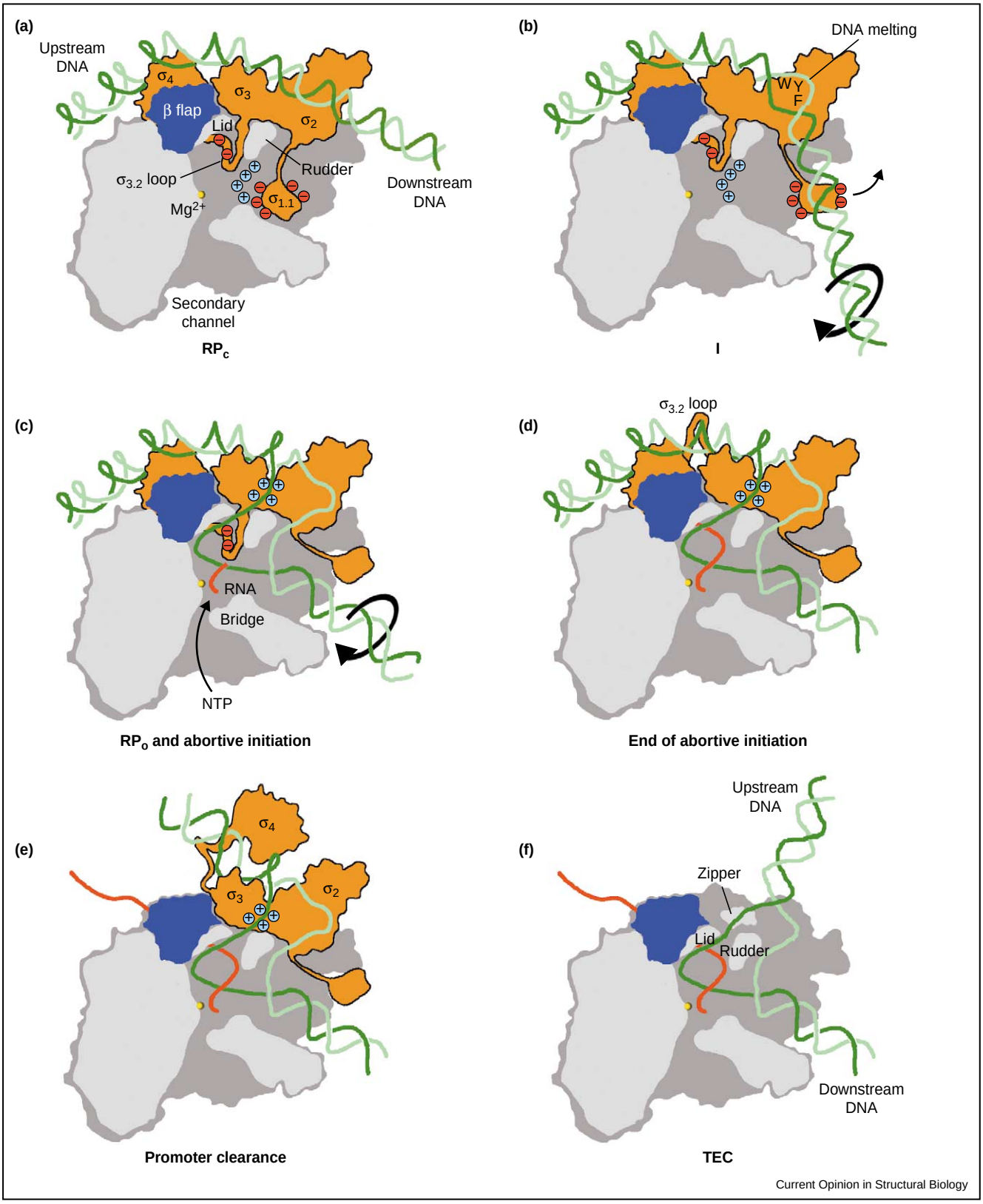
The recent RNAP holoenzyme structures do not provide direct structural information on the role of $\sigma_{1.1}$, because this domain is absent in the Taq holoenzyme structure (an N-terminal truncation mutant of Taq σ^A was used to obtain crystals) [13••] and is disordered in the Tth holoenzyme [15••] and in the complex of the Taq holoenzyme and fork-junction DNA [14••]. A fluorescence resonance energy transfer study of the *E. coli* holoenzyme has provided direct evidence that $\sigma_{1.1}$ is positioned inside the RNAP active-site channel of holoenzyme. After formation of the promoter open complex, $\sigma_{1.1}$ is displaced outside the channel [16•], which explains how $\sigma_{1.1}$ can affect the kinetics of open complex formation. It was proposed that the positioning of $\sigma_{1.1}$ in the RNAP channel may widen the channel to facilitate the entry of double-stranded DNA (Figure 3) [13••], but elucidation of the precise role of $\sigma_{1.1}$ awaits further studies.

The σ_2 domain

Genetic studies indicate that region 2.4 recognizes the promoter −10 element (probably in double-stranded form; reviewed in [7]). Region 2.3 is essential for DNA melting through single-strand, sequence-specific binding to the nontemplate strand of the −10 element [27], which stabilizes the initial transcription bubble.

Although the molecular details of the interaction between σ and promoter DNA were not resolved in the 6.5 Å resolution complex of the Taq holoenzyme with fork-junction DNA, the residues of σ region 2.4 are positioned within reach of the −12 position (the only double-stranded portion of the −10 element), and several highly conserved aromatic residues in region 2.3, which is important for the melting function [28–30], are positioned to interact with the exposed bases of the −10 element nontemplate tail of the fork-junction DNA. Probably of most importance, a tryptophan residue, which is universally conserved in group 1 σ factors [17], is positioned to stack on the exposed face of the −12 position, forming the upstream edge of the transcription bubble [14••].

Figure 3



Structural transitions during the steps of transcription initiation. Shown are cross-sectional views of the RNAP holoenzyme (β flap, blue; σ, orange; rest of RNAP, gray; catalytic Mg^{2+}, yellow sphere), promoter DNA (template strand, dark green; nontemplate strand, light green; −10 and −35 elements, yellow) and the RNA transcript (red) at the RP_c **(a)**, intermediate (I) **(b)**, RP_o and abortive initiation **(c)**, end of abortive initiation **(d)**, promoter clearance **(e)** and TEC **(f)** stages of transcription initiation. The view is looking down on top of the β subunit, but with most of β removed, revealing the inside of the RNAP active site channel.

The σ_3 domain and $\sigma_{3.2}$ loop

The σ_3 is a compact domain of three α helices. Amino acids on the N-terminal helix (region 3.0) are involved in recognition of the extended −10 element [31,32], thereby stabilizing the open complex [33].

A C-terminally truncated variant of σ, lacking both the $\sigma_{3.2}$ loop and σ_4, retains weak transcription activity on extended −10 promoters, which can be increased to wild-type levels by increasing the concentration of initiating nucleotide [12•]. The proximity of the $\sigma_{3.2}$ loop to the active site suggests that removal of this loop is the origin of the defect in binding initiating nucleotide. Whether this is a direct or indirect effect remains to be determined.

The σ_4 domain

The σ_4 domain (region 4.1–4.2) is C-shaped, with a concave pocket coated with hydrophobic residues of region 4.1. Mutations in some of these residues result in defects in binding to the core RNAP [34]. In the holoenzyme, the β-flap-tip helix fits into the σ_4 pocket. The σ_4 domain clamped to the end of the β flap forms the σ_4-flap mobile module. The bend in the DNA at the −35 element induced by σ_4 binding [12•] alters the trajectory of the upstream DNA (as shown in the models of Figure 3), bringing it closer to the RNAP and facilitating interactions between the α C-terminal domain and upstream DNA [35] and interactions with activators that bind upstream of the −35 element.

The pathway from initiation to elongation

The interaction of the RNAP holoenzyme with promoter DNA initiates a series of structural transitions beginning with the initial closed promoter complex (RP_c) and culminating with the processive TEC. The structure of the complex between the Taq RNAP holoenzyme and fork-junction DNA provides a starting point for models of distinct structural steps in the pathway. Our current view of how RNAP progresses although this pathway is illustrated in Figure 3 and described below.

RP_c

In RP_c, the double-stranded −35 and −10 elements interact with σ_4 and σ_2, respectively (Figure 3a). The holoenzyme protects promoter DNA from DNase I or hydroxyl radical cleavage across a region extending from roughly the −54 to the −6 position [36–39]. But there is no protection downstream of the −6 position, consistent with a continuous DNA double helix. Also shown in Figure 3a are the $\sigma_{3.2}$ loop, which protrudes into the RNAP active-site channel (the active site is marked by the Mg^{2+}) and out underneath the β flap, and $\sigma_{1.1}$, which is positioned in the active-site channel through electrostatic interactions.

Intermediate steps

We speculate that the conserved aromatic residues of σ region 2.3 are perfectly positioned to take advantage of transient exposure of the nontemplate strand bases of the −10 element; this exposure arises from the natural 'breathing' or distortion of the AT-rich −10 element [40]. These interactions between the protein and single-strand DNA would stabilize an initial, short segment of melted DNA, corresponding to the upstream edge of the final transcription bubble. This melting would be associated with unwinding of the DNA (Figure 3b, circular arrow) and would create flexibility in the DNA at the bubble, allowing the downstream DNA to bend or kink across the entrance of the active-site channel (Figure 3b), as proposed previously [15••,41]. The process of DNA entry into the RNAP channel must be accompanied by simultaneous exit of $\sigma_{1.1}$ [13••,16•] by an unknown mechanism.

RP_o and abortive initiation

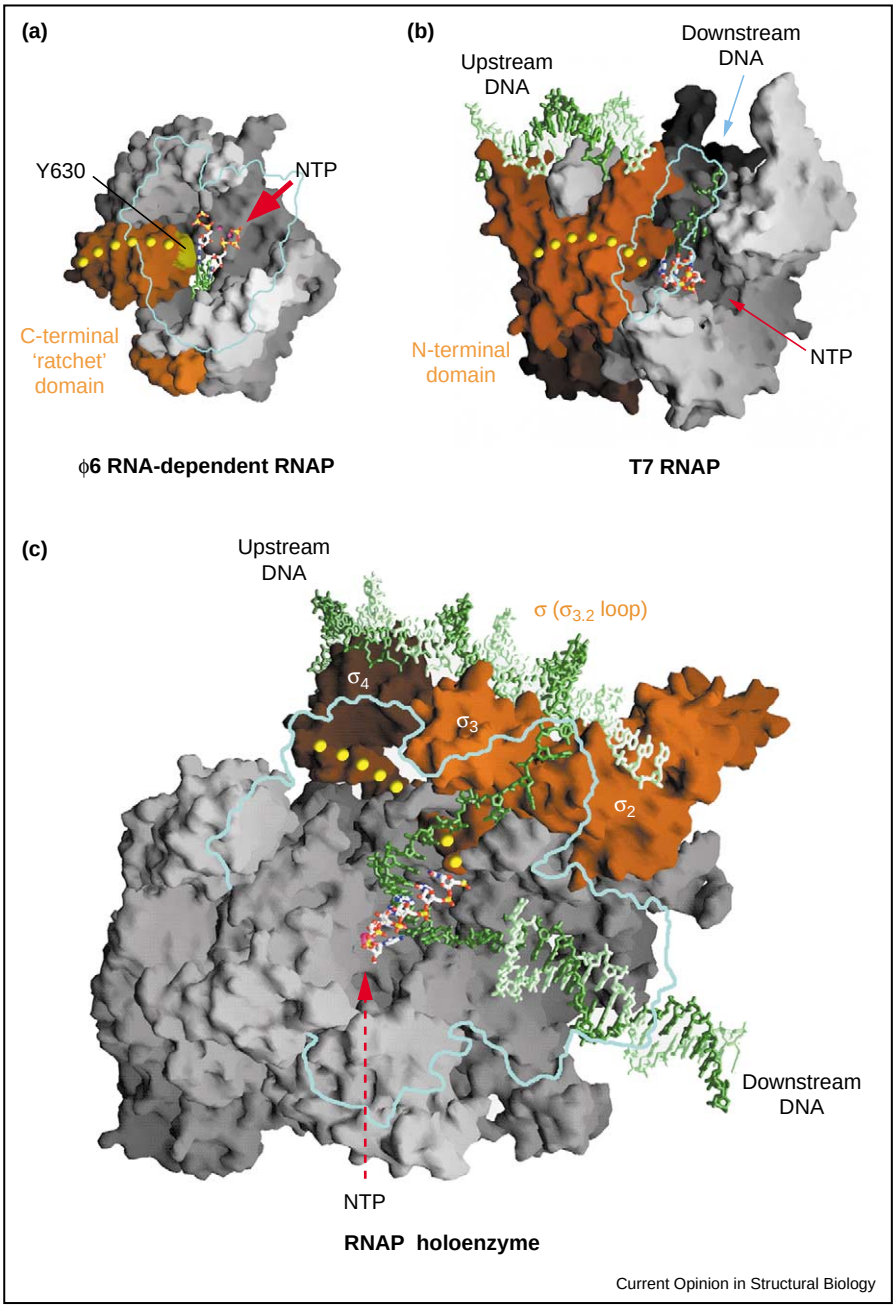
In the final open RNAP–promoter complex (RP_o; Figure 3c), DNA melting extends downstream past the transcription start site (+1) to complete the transcription bubble. The single-stranded template strand is directed to the active site through a positively charged tunnel, which is completely enclosed on all sides by protein [14••]. The downstream, double-stranded DNA from the +5 to about the +12 position is clamped inside another protein tunnel between the β and β′ subunits; this location is consistent with footprinting and functional studies demonstrating the importance of this DNA to the stability of the complex [38,39,42–44].

On formation of RP_o, the RNAP active site, which is provided with NTP substrates through the secondary channel, begins catalyzing the synthesis of an RNA chain. A transcript that is only a few nucleotides in length, however, will encounter the $\sigma_{3.2}$ loop in its path (Figure 3c), beginning the process of abortive initiation [13••]. At each step, the elongating RNA chain must either displace the $\sigma_{3.2}$ loop out of its path, or else dissociate from the complex and be released (probably through the secondary channel). Eventually, the RNA chain elongates to a length of about 12 nucleotides, which is sufficient to fill the RNA–DNA hybrid and upstream RNA exit channel completely under the β flap, thereby displacing the $\sigma_{3.2}$ loop (Figure 3d) and marking the end of abortive initiation.

Promoter escape

The displacement of the $\sigma_{3.2}$ loop may couple the presence of an RNA chain of about 12 nucleotides to the initial stages of promoter escape by destabilizing interactions between σ_4 and the β flap (Figure 3e). Release of σ_4 from the β flap would, in turn, destabilize interactions between σ_4 and the −35 element, allowing the RNAP to 'let go' of the promoter and translocate downstream as it elongates the RNA. This transition into elongation does not require the complete release of σ, as has been appreciated from experimental observations [45–48], because the binding of σ_2 and σ_3 to the RNAP is not

Figure 4



Initiation complexes of three RNAPs. Shown are the molecular surfaces of three RNAPs capable of primer-independent initiation, along with the nucleic acids observed (a,b) or modeled (c) in each structure. Obscuring features of each structure have been removed (outlined in light blue). The nucleic acids are colored dark green for the template strand, light green for the nontemplate strand, and white for the RNA product. The protein structures are colored gray, apart from the structural element that blocks the path of the elongating RNA product, which is orange. The eventual path of the full RNA product is indicated by the yellow dots. The red arrow indicates the path for incoming nucleotide substrates to enter the active site. **(a)** ϕ6 RNA-dependent RNAP initiation complex [52••]. The palm domain has been removed for clarity (blue line). The C-terminal 'ratchet' domain is colored orange. Tyr630, which makes direct interactions with the initiating nucleotide substrate, is highlighted in yellow. **(b)** T7 RNAP initiation complex [54]. The thumb domain has been removed for clarity (blue line). The N-terminal domain is colored orange. **(c)** The Taq RNAP holoenzyme bound to fork-junction DNA, with modeled DNA and RNA corresponding to an initial transcription complex at a promoter [14••]. The β subunit has been removed for clarity (blue line). The σ subunit is colored orange. Generated by grasp [57].

incompatible with the paths of the nucleic acids in the TEC [11,49–51]. Eventually, complete release of σ results in the TEC containing the core RNAP (Figure 3f).

Conclusions

In addition to the insights described above, comparison of the multisubunit cellular RNAP initiation process with other RNAPs capable of primer-independent initiation leads to some surprising generalizations. In ϕ6 RNA-dependent RNAP (Figure 4a), the C-terminal 'ratchet' domain blocks the path of the elongating RNA product and is proposed to swing out of the way after the synthesis of a product trimer to form the elongation complex [52••]. Tyr630 on the ratchet domain faces the active center and stabilizes the binding of the initiating nucleotide substrate [53], which enters from the opposite direction.

In the T7 RNAP initiating complex (Figure 4b), the N-terminal domain blocks the path of the elongating RNA transcript, leaving room for only a trimeric product [54]. NTP substrates also enter through a channel from the opposite direction. Recent structures show that the enzyme undergoes considerable conformational rearrangements on entering elongation, resulting in the loss of the promoter-binding site (promoter clearance) and the creation of a binding site for the RNA product [55••,56••].

Thus, in ϕ6 and T7 RNAPs — as in the RNAP holoenzyme (Figure 4c) — protein elements block the path of the elongating RNA product at the level of 2–3 nucleotides. Large conformational rearrangements (of the C-terminal ratchet domain in ϕ6 RNAP, of the N-terminal domain in T7 RNAP, or of the $\sigma_{3.2}$ loop in RNAP holoenzyme) are required to accommodate longer product chains. These conformational changes mark the transition from the initiation to the elongation phase, and may act as signals for initiation-specific regulatory factors to disengage or for elongation-specific regulatory factors to engage. For T7 RNAP and the RNAP holoenzyme, the changes that accompany elongation of the RNA product beyond a certain length also initiate the process of promoter clearance by weakening promoter-specific interactions.

Finally, elements of the ϕ6 RNA-dependent RNAP ratchet domain have a direct role in stabilizing the binding of the initiating nucleotide substrate, just as the RNAP holoenzyme $\sigma_{3.2}$ loop seems to do (at least indirectly). This function assists primer-independent initiation but is not required after the formation of the first phosphodiester bond, when the corresponding site is occupied by the 3′ end of the RNA product chain. Thus, convergent evolution seems to have solved the problems of primer-independent initiation, weakening promoter interactions to allow entry into elongation and signaling the transition to elongation in conceptually similar but structurally unrelated ways.

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