The structure of bacterial RNA polymerase

Kati Geszvain* and Robert Landick†

Transcription by RNA polymerase (RNAP) is the central control on the flow of genetic information. By selecting which RNAs are made, RNAP dictates how cells adapt to new environments, interact symbiotically or pathogenically with hosts, respond to stress and starvation, and multiply. RNAP accomplishes this task by deciding where and how often to start transcription, how to elongate RNAs, and where to stop. Recently reported crystal structures of RNAP have begun to shed light on this central enzyme of gene expression, revealing a remarkable molecular machine whose complex structure begins to explain the fundamental mechanisms of transcriptional regulation.

In this review, we will describe recent advances in RNAP structure and their implications for understanding the mechanism of transcription and the regulation of key steps in the transcription cycle. To lay a foundation for understanding the structures, we begin with a summary of the main features of the transcription cycle, RNAP’s mechanism, and RNAP’s subunit composition and primary structure.

The transcription cycle

There are three main steps in the transcription cycle: initiation, elongation and termination. During initiation, the core RNAP enzyme (subunit composition α2, β', β and ω in bacteria; reference (13)) binds to one of the family of σ initiation factors. The resulting holoenzyme is able to bind specifically to the promoter DNA, forming the closed complex (RPo; references 14, 47) in a process called promoter DNA, forming the closed complex to the template strand (78). RP o is capable of DNA have melted to allow active-site access to the RNAP's mechanism, and a summary of the main features of the cycle.

The structure of RNAP

A summary of the main features of the transcription cycle begins to explain the fundamental mechanisms of transcriptional regulation.

Conservation of RNAP primary sequences

The basic architecture of multi-subunit RNA polymerases is conserved throughout the living world, with two large subunits forming the bulk of the enzyme (β' and β in bacteria), a homo- or hetero-dimer of smaller subunits on the periphery of the enzyme involved in assembly (the α dimer in bacteria), and at least one accessory subunit (ω in bacteria). β' and β are split into two polypeptides in some organisms (84) and can be fused into one polypeptide in others (106). Together, β' and β form the catalytic core of the enzyme and maintain the nucleic acid scaffold of the TEC (Fig. 1B and 2A), β' and β are homologous to the two largest subunits of eukaryotic RNAPs (RPB1 and RPB2, respectively in yeast RNAP II). Elements of sequence similarity are present in a conserved order in the primary structure of these subunits: A through H in β' and A through I in β (1, 93). In the three-dimensional structure of core RNAP, these conserved elements cluster around the active center, with the more divergent regions of the subunits located on the periphery of the enzyme (see Fig. 6 in reference 109).

The two α subunits play non-equivalent roles in the core structure (109). One α monomer, referred to as αL, contacts exclusively the β subunit (green in Fig. 2A), whereas the other contacts β' (eII, yellow in Fig. 2A). This is consistent with the order of assembly of the subunits into RNAP: the α dimer forms first, then β and β' bind consecutively (40). The α subunits are divided into two functional domains connected by a

†To whom correspondence should be addressed.

E-mail: landick@bact.wisc.edu
CTD is related to the helix-hairpin-helix (HhH) family of DNA binding proteins (76). The α subunit, which may also function in RNAP assembly, is homologous to RPB6 in eukaryotic RNAP II (62).

Currently, crystal structures are available for core RNAP (22, 109), holoenzyme (66, 97), holoenzyme with a “fork junction” DNA that mimics elements of the open complex (65), and the TEC (34). From the crystal structures and biochemical and genetic evidence, models of some intermediates in the transcription cycle for which there are no structures available have also been proposed (45, 65). Both the crystal structures themselves and the models derived from them are necessarily static approximations of the conformations assumed by RNAP during the transcription cycle. When viewed with appropriate caution, however, they afford powerful insight into the behavior of this intriguing enzyme. Some of these insights have been discussed in detail in recent reviews (8, 21, 29, 39, 81, 105). In this chapter, we will provide a comprehensive overview of what has been learned from the RNAP structures and models.

### Core RNAP

#### Structural conservation

The elucidation of the structure of RNAP from multiple organisms has revealed that the structure of RNAP, as well as its sequence, is conserved among prokaryotes and eukaryotes. The X-ray crystal structure of *Thermus aquaticus* RNAP aligns well with a 15 Å cryo-EM structure of *E. coli* RNAP (23), supporting the idea that the thermophilic and mesophilic RNAPs have similar structures. Therefore, biochemical data derived from work with *E. coli* can be used in conjunction with structural data from *T. aquaticus*. The overall shape is the same for both yeast and bacterial RNAP, as are discrete elements of the structure. These structural motifs, however, do not correspond to the elements of sequence conservation (β A – I, β’ A – H). Although the names of the conserved sequence elements remain in use, descriptive names such as the rudder or bridge helix (defined below) are needed to identify structural motifs (22, 109).

#### Overall structure

The structure of *Thermus aquaticus* core RNAP, solved in 1999, first revealed the general shape of the enzyme (109). RNAP is 150 Å by 115 Å by 110 Å; with a deep cleft 27 Å wide that creates an overall “crab-claw” shape (Fig. 2A, inset). The β and β’ subunits interact extensively, with part of the β subunit

---

**Fig. 1.** Model of RNAP structure and function. (A) The transcription cycle. Core RNAP is blue, the initiation factor α is orange, the DNA template is black and the nascent RNA transcript is red. (B) Model of the TEC. The incoming NTP and an arrow showing its path into the TEC through the secondary channel are green. (C) The nucleotide addition cycle. The i and i + 1 sites are shown as two joined circles. Inset: two Mg^{2+} ions (red circles) catalyze phosphodiester bond formation between the 3’ end of the nascent transcript and an incoming NTP.
Fig. 2. Structure of core RNAP (A) The downstream face of core RNAP. The model is based on the coordinates of the T. thermophilus holoenzyme (PDB ID 1I6V; reference 109), with the α subunit and a non-conserved region not present in E. coli β′ (aa 164-448) removed and the RNAP conformation adjusted to that observed in the core T. aquaticus RNAP (PDB ID 1I6V; reference 109) by movement of RNAP mobile modules (23). Sequence insertions present in E. coli (23) are not depicted. The path of the secondary channel is illustrated by a dashed line. The α-carbon backbone is shown as a worm inside a semi-transparent surface. Subunits are color-coded as follows: β′, pink; β, cyan; α, green; αII, yellow; ω, grey. The β′ bridge helix and trigger loops are depicted as green and orange worms, respectively. Zn\(^{2+}\) and Mg\(^{2+}\) atoms are depicted as yellow balls. The α CTDs are shown in arbitrary positions 43 Å from core. They are shown as isolated domains, but may be present as a dimer in RNAP (7). The boxed inset depicts the upstream face of core RNAP, illustrating the “crab-claw” shape of the enzyme. (B) The active-site channel. The RNAP model in A is shown rotated 90° to the right. The subunits are shown as solid surfaces except the ZBD, β′ Mg\(^{2+}\)-binding loop, rudder, lid and zipper are shown as pink worms in a semi-transparent surface and the β flap domain is shown as a dark blue worm in a semi-transparent surface. The β′ bridge helix is depicted as a green worm. The antibiotic rifampicin is depicted in red (β is rendered semi-transparent in front of Rif to reveal the antibiotic nestled in its binding pocket.) The clamp, protrusion and lobe are outlined in black. (C) Two αβCTDs bound to UP element DNA. This model is based on the crystal structure of αCTD, DNA and catabolite activator protein (PDB ID 1LB2; reference 6). Non-template DNA is light green; template DNA is dark green. Two residues involved in recognition of the UP element (Arg265 and Asn294) are shown as red sticks. The CAP interaction determinant is indicated by the blue space-fill valine residue at position 287. Asp259 and Glu261, two residues that interact with the region 4, are shown as orange sticks. Only one of each symmetric pair of residues is labeled.

Active-site channel

The active-site (or main) channel, formed by the cleft between β and β′, is highly conserved among RNAPs and is lined with
structural elements essential for catalysis and maintaining the nucleic-acid scaffold. The active center is marked by a Mg$^{2+}$ ion chelated at the base of the channel by three aspartate residues (from the universally conserved NADFDGD motif of β′ region D (Fig. 2B, Mg$^{2+}$ I). Closing over the i + 1 site is a loop called the βD loop II, centered on E. coli residue 568 (most easily seen in Fig. 5B). This loop is immediately adjacent to the β′ bridge helix, which lies just downstream of the i + 1 site (Fig. 2A and B). The binding pocket for the antibiotic rifampicin (Rif, defined by RifR substitutions and an RNAP-Rif co-crystal; reference 18) is centered approximately 20 Å upstream from the active center on the wall of the active-site channel (Fig. 2B). Rifampicin positioned in this pocket would block growth of the RNA chain past 2 or 3 nucleotides, explaining the bactericidal effect of the antibiotic (18). Further upstream along the active site channel is a “figure 8” shaped loop called the β′ rudder. The upstream edge of the active site channel is formed by the β subunit’s flap domain, as well as the β′ lid and zipper domains (109).

The secondary channel
Immediately downstream of the active site, the bridge helix separates the main channel into a downstream DNA entry channel and a 10-12 Å wide secondary channel (Fig. 2A and B). Just inside the secondary channel lies the β′ trigger loop, which is partially disordered in T. aquaticus core and RNAP II, but ordered in the T. thermophilus holoenzyme. This channel is too narrow for double-stranded nucleic acids to pass through, but is optimally positioned to allow NTP’s access to the active center. Therefore, it has been proposed that the secondary channel serves as the entry site for NTPs (109). In a backtracked elongation complex, in which the RNAP has moved backward along the DNA and RNA placing the active site over an internal phosphodiester bond, the 3′ end of the RNA transcript inserts into the secondary channel (30).

The RNA-exit channel
After the nascent transcript separates from the RNA:DNA hybrid, it is extruded from the TEC through the RNA-exit channel (Fig. 2B and 5A). Cross-linking data suggest that the flap covers this channel (45), with the RNA passing between the base of the flap and the β′ lid (96). It has been suggested that during elongation the flap is closed down around the RNA in the exit channel, possibly contacting the β′ ZBD, but that hairpin-dependent pause signals the formation of an RNA hairpin underneath the flap opens the RNA exit channel by clamp or flap movement, causing an allosteric change in the active site that alters the elongation behavior of the enzyme (96).

The downstream DNA channel
The downstream DNA is held in a channel formed by the β′ lobe and the β′ jaw, with 15 – 20 bp in the TEC protected from premature cleavage (Fig. 2B and 5A). At least 9 bp of duplex DNA downstream of the active site is required for TEC stability (69), and the sequence of the downstream DNA can modulate the response to pause (53) and termination signals (74, 94), as well as modulate the rate of elongation (38). This suggests the interaction between the downstream DNA and RNAP is important for TEC function.

αCTD
Although the αCTD is not resolved in any of the RNAP crystal structures, structures are available of the isolated domain. An NMR structure is available for the isolated domain from E. coli (41) and a 3.1 Å X-ray co-crystal is available of E. coli αCTD with the catalytic activator protein (CAP) and an UP element (6). These structures reveal that the αCTD is a compactly folded domain with four α helices and one non-standard helix. Four of these five helices are involved in forming two helix hairpin helix (HHH) motifs, identifying the αCTD as a member of the HhH family of DNA-binding proteins (Fig. 2C). This family is characterized by the presence of two anti-parallel α helices connected by a hairpin loop (76).

During transcription initiation, the αCTD makes multiple functional interactions, with the DNA in an UP element, activators such as CAP and, potentially, σ region 4. In a co-crystal of αCTD, DNA, and CAP, Arg265 and Asn294, located at the helix-hairpin junctions of the two HhH motifs in the αCTD, position each other in the narrowed minor groove of three adjacent A/T base pairs in the UP element, with Arg265 making a base-specific contact to N3 of an adenine in the sequence (Fig. 2C). This interaction is aided by contacts of several other αCTD side-chains to phosphates along the narrowed minor groove. In the co-crystal (6), αCTD and CAP interact through Activating Region 1 (AR1) of CAP and one of the surfaces of the αCTD available for interaction with transcriptional activators, the 287 determinant (Val287), as was predicted from genetic experiments (80). When the CTD is bound to a promoter-proximal UP element or interacts with an activator that positions it next to the promoter (i.e. a class I CAP site), it is immediately adjacent to the binding site of σ region 4.2. A model of a DNA-αCTD-σ region 4.2 ternary complex assembled from the T. aquaticus σ region 4-DNA complex (19) and the E. coli αCTD-DNA complex identifies surfaces of α and σ that lie in close proximity (Fig. 2C). This suggests that an interaction between the CTD and σ is involved in transcription activation, possibly by stabilizing σ region 4’s contact to the -35 promoter element. In agreement with this, substitutions generated at these surfaces in the αCTD at Asp259 and Glu261 and σ region 4.2 at Arg603 can decrease UP element function (20, 77).

Holoenzyme
The σ$^{70}$ family of initiation factors
The multiple members of the σ initiation factor family are divided into two classes, with little sequence conservation between the two. One class is similar to E. coli’s “housekeeping” σ, σ$^{70}$. The other is similar to σ$^{32}$ or σ$^{54}$, which is responsible for transcribing genes required for nitrogen fixation as well as the stress response (12). The σ$^{70}$ class of initiation factors is the better characterized of the two. It is composed of the primary σ factors, which are responsible for transcribing most genes involved in basic cellular metabolism, and the alternative σ factors, which transcribe subsets of genes required under specific growth conditions, such as heat shock, or specific cellular processes, such as flagella production (57, 79). The primary σ’s have four regions of sequence conservation (1.1-1.2, 2.1-2.4, 3.0-3.2, and 4.1-4.2, Fig. 3A) that are responsible for core binding, promoter recognition and DNA melting as well as a non-conserved region inserted between regions 1.2 and 2.1 that is only found in some σ’s (57, 85). Region 1.1 also is not present in the alternative σ factors (57).

No complete structure for free σ is available, but biochemical data and X-ray crystal structures of proteolytic fragments provide some insight into the structure of the subunit. Limited proteolysis of free σ indicates that the subunit is made up of compactly folded domains joined by flexible linkers (85). This has been confirmed by the publication of the crystal structure of portions of the housekeeping σ, σ$^{70}$, from T. aquaticus (19). Fortuitous contamination of the crystallization solution with a protease produced fragments of σ containing regions 1.2 through 3.1 and regions 4.1 to 4.2. In this structure, region 1.2 through 2.4 folds into a compact structure, with a flexible linker joining it to region 3. What had been referred to as conserved region 2.5 (5) is actually part of the region 3 domain; therefore it has been renamed region 3.0. Regions 4.1 and 4.2 also fold into a compact structure (Fig. 3A). Region 1.1 and the linker between regions 3 and 4 were completely proteolysed and therefore are not visible in this structure. Region 1.1 blocks the ability of region 4.2 to bind to the -35 element of the promoter (25, 26), suggesting that these two regions interact in free σ. However, NMR studies with region 4.2 detect no interaction
between it and region 1.1 (17). Therefore, the structure and location of region 1.1 in free σ remains unknown.

**σ-core interactions**

The crystal structures for holoenzyme from *T. aquaticus* and *T. thermophilus* reveal the interactions between σ and core that confer on RNAP the ability to recognize the promoter (66, 97). These interactions are quite extensive, as had been predicted by biochemical studies (8, 35, 86). In holoenzyme, σ is folded into three flexibly linked domains, σ₂, σ₃ and σ₄ (Fig. 3B), containing conserved regions 1.2 – 2.4, 3.0 – 3.1, and 4.1 – 4.2, respectively (66). σ₂ is bound to the β′ clamp, with the major contact between σ region 2.2 and the coiled-coil domain of β′ (Fig. 3A), in agreement with biochemical and genetic evidence that these two regions are the primary interface between core and σ (3). σ₂ is located within the active-site channel, contacting primarily the β subunit near the active site. σ₄ wraps around the flap-tip helix of the β flap domain; a hydrophobic patch on the flap-tip helix thus becomes buried in the hydrophobic core of σ₄. Residues in regions 2.4, 3.0 and 4.2 that have been identified as making contacts with the promoter DNA are all surface exposed on the holoenzyme structure (Fig. 3B).

The compact domains of σ are connected by flexible linkers that allow the subunit to stretch across the upstream face of the enzyme. The short linker between σ₂ and σ₃ is highly conserved, and interacts with the β′ zipper (Fig. 3B), a conserved structural feature also present in eukaryotes. The 45 Å distance between regions 3 and 4 is spanned by conserved region 3.2. The 3.2 linker is almost completely buried in the active-site channel and RNA-exit channel (Fig. 3C), first interacting with the β′ rudder, zipper and lid before turning toward the active center (a 9-residue segment of the linker approaches within ~25 Å of the active-center Mg²⁺ I), then turns back to enter the RNA-exit channel. In the relatively closed holoenzyme structure, the β′ lid contacts the inner surface of the β flap, completely surrounding the 3.2 linker (66). The extended conformation of σ across core positions regions 2.4 and 4.2 optimally to bind the –10 and –35 promoter elements spaced 17 bp apart (51).

The position of region 1.1 in the holoenzyme is uncertain, but the available data suggest that it is located in the downstream side of the active-site channel (60). Neither holoenzyme crystal structure includes region 1.1; it has been proteolyzed in the *T. aquaticus* structure (66) and is not resolved in the *T. thermophilus* structure (97). Some evidence has suggested that region 1.1 is bound at the upstream face of RNAP, interacting with the flap and σ region 4 (10, 35). However, if this interaction occurs it must be a transient intermediate in holoenzyme formation since fluorescence resonance energy transfer experiments that mapped the contacts between core and σ showed that region 1.1 is located within the downstream DNA channel in holoenzyme (60). Also, in the holoenzyme crystal structure (66), the amino-terminal...
fragment of σ is pointed into the active-site channel, not toward the flap (Fig. 3B).

Conformational changes in holoenzyme formation

Upon holoenzyme formation, both the core subunits and σ undergo conformational changes. In the core subunits, some regions move, whereas others that were disordered in the core RNAP structure become ordered (66). In the T. aquaticus holoenzyme structure, the β clamp and the δ lobe domains rotate in towards the active-site channel, narrowing the width of the channel by 10 Å relative to core. The positioning of σ domains 2 and 3 on opposite sides of the active-site channel, with σ2 on the mobile clamp domain, suggests σ could play a role in opening and closing the active-site channel during promoter binding. The interaction with σ region 4 rotates the flap-tip helix about 15° towards the non-template strand relative to core. The β protrusion domain rotates about 10 Å out from the active site channel, most likely in response to the changes in the other domains (Fig. 3B). The β ZBD, lid and zipper domains that were disordered in the core structure all become ordered and visible in the holoenzyme (66, 97).

The DNA-binding ability of σ is unmasked upon binding to core by moving region 1.1 and changing the conformation of the DNA-binding domains themselves. The distances between region 1 and region 2, and region 4 and region 2 increase upon core binding, suggesting the conformation of σ in holoenzyme is “stretched out” relative to free σ and region 1.1 has been moved away from the DNA-binding surfaces of region 2.4 and 4.2 (15, 16). A fragment of σ containing part of conserved region 1.2 through to region 2.4 binds specifically to the non-template strand of the promoter DNA only after binding to β’ or a fragment of β containing the coiled-coil domain, suggesting the interaction between σ and core results in a conformational change within region 2 that allows DNA binding (104). In the holoenzyme structure, two rearrangements in σ region 2 are evident. First, a loop that covers the core binding surface in region 2.2 moves out of the way. Secondly, the bundle of helices made up of regions 1.2 and 2.1 - 2.4 rotates about 12° relative to the non-conserved region. However, it is not clear how these changes facilitate DNA binding. Possibly, further conformational changes occur in region 2 during the process of RPo formation that enable non-template DNA binding or some other interaction inhibits DNA binding by free σ or the σ fragment (2).

Promoter Recognition

Closed complex formation

Transcription initiation is a multi-step process in which holoenzyme (R) binds to the promoter (P) to form the closed complex (RPc), then this complex undergoes an isomerization through at least one intermediate (RPi) to the open complex (RPo) that is capable of binding NTPs and initiating transcription. The steps in this reaction can be depicted as (78):

\[ R + P \leftrightarrow RPi \leftrightarrow RPo \rightarrow ITC \]

In the RPo σ^{70}-containing holoenzyme engages the -10 and -35 conserved hexamers of the promoter, with the DNA remaining double-stranded and protected from both nuclelease and hydroxyl radical cleavage from -54 to -6 (47). The spacing between the -10 and -35 elements can vary, but is almost always between 16 to 18 bp (37). A separate set of σ^{70}-dependent promoters lacks the -35 element and instead requires recognition of the -16 TG element (A TRTGn motif located immediately upstream of the -10, also referred to as the extended -10; reference 11). The structure of the RPc must accommodate these variable DNA contacts as well as facilitate the transition to the RPo.

Although no crystal structure of RPo exists, its structure can be modeled from the holoenzyme structure, as well as from a σ2- DNA co-crystal (19) and the holoenzyme-fork junction structure (described below; reference 65). In RPc, the DNA-binding elements of σ lie across one face of the holoenzyme, defining this as the upstream face (Fig. 4A). The -10 recognition helix of region 2.4 is spaced about 16 Å away from the -16 TG element recognition helix in region 3.0, which would easily accommodate the 5 bp of DNA.

**Fig. 4.** A model for RPc formation (adapted with authors’ permission from Fig. 3 of reference 64) (A) The holoenzyme depicted as in Fig. 3B, rotated to show the upstream face (the same as in the inset to Fig. 2A) and with σ shown as an orange surface. The gate loop is shown as a blue worm and σ1.2 helix is red (lies behind σ1 in this view). The line across the RNAP depicts the plane at which RNAP would be cut to generate the view shown in the B - D. (B) A cut-away view of RPc. Core is grey, σ is orange and the flap is blue. The DNA is dark and light green and lies along the surface of the upstream face of RNAP. The negatively charged σ region 1.1 lies in the positively charged downstream-DNA channel. The β lobe and gate loop would lie above the plane of the page, protruding into the downstream DNA channel above σ region 1.2. (C) RPc, a possible intermediate in RPo formation. A kink forms in the DNA within the -10 element. Aromatic residues in σ region 2.3 interact with the non-template strand to assist DNA opening. The DNA moves into the downstream-DNA channel, replacing region 1.1. The β gate loop prevents entry of double-stranded DNA into the active site and may assist in unwinding the DNA. (D) RPo. The downstream DNA is inserted in the downstream-DNA channel and melting has extended from the -10 element to the transcription start site. The template strand is guided into the active-site channel by positive residues in σ regions 2.4 and 3.0. NTPs entering through the secondary channel can be incorporated into a nascent transcript (red), extension of which is blocked by the σ3.2 loop, resulting in abortive initiation.
separating the two elements (17 Å spacing, assuming straight, B-form DNA.) Two residues in σ region 3.0 previously identified genetically to be involved in the recognition of the -16 TG sequence face into the major groove of the -16 TG element (5). Region 4.2 is located on the β flap domain 76 Å away from region 2.4. This position could accommodate the canonical 17 bp spacing between the -10 and -35 elements with an 8° bend centered at about -25 bp. However, the flap-σ region 4 complex can move relative to the DNA by at least 6 Å, allowing holoenzyme to bind promoters with a non-canonical spacing (66, 97). The σ-α-DNA co-crystal shows that region 4.2 interacts with the major groove throughout the -35 element, contacting the phosphate backbone as well as making specific interactions with the DNA bases. The insertion of region 4.2’s HTH recognition helix into the major groove induces a 36° bend in the DNA; this distortion may be important for the interaction of transcriptional regulators that bind upstream of the -35 (19). The DNA in RP 0 does not enter the active-site channel, explaining the lack of nuclease protection downstream of -5 in this complex (Fig. 4A).

Open complex structure

Before RNAP can initiate transcription, the downstream DNA must insert into the downstream DNA channel, the DNA around the start site must be melted and the template strand must insert into the active site to form the open complex (RP 0). A crystal structure of holoenzyme with a fork junction DNA template has been used to model this complex (65). The fork junction DNA contains double-stranded DNA from -41 through the -35 element up to the first base pair of the -10 element (the -12 bp), and the single-stranded non-template strand of the -10 element from -11 through to base -7. Holoenzyme bound to the fork junction DNA (RF) mimics many properties of the RP 0: (i) RF, like RP 0, is resistant to DNA binding competitors, (ii) substitutions in the promoter or RNAP that inhibit RP 0 formation also inhibit binding of the fork junction DNA, and, (iii) formation of RF is a multi-step process and some of the intermediates are similar to those in RP 0 formation (33, 36). Therefore, the RF structure can be used to model the structure of the RP 0 (65).

The RF structure suggests many details of the RP 0 structure. The DNA lies along one face of the RNAP, with the 8° bend at about nt -25 and a sharp, 37° turn at about -16 pointing the DNA towards the active center. In the RF structure, all of the RNAP-DNA contacts with the consensus promoter elements are mediated by σ. The clamp domain, along with σ region 2 bound to it, rotates in toward the main channel, causing it to close by 3 Å relative to the holoenzyme. The flap domain also moves about 6 Å downstream relative to the DNA, illustrating the flexibility of this domain. However, the σ region 4-DNA interaction appears to be distorted by crystal packing forces so the positions of the flap, region 4, and -35 DNA in this crystal likely does not represent their normal positions. Residues in region 2.4 of σ identified as required to recognize the -12 position of the -10 element (89) are surface exposed and contact the -12 base (Fig. 3A). In σ region 2.3, highly conserved aromatic residues that play a role in promoter melting (71) are surface exposed and positioned to interact with the single-stranded non-template DNA. One of these residues, Trp256, appears to be stacked on the exposed face of the base pair at position -12. This interaction likely forms the upstream edge of the transcription bubble and its formation may be the defining step in DNA melting (56). Universally conserved positive residues in regions 2.2 and 2.3 that appear to be involved in DNA binding and open complex formation (95) are positioned to interact with the negatively charged phosphate backbone of the non-template strand immediately upstream of the -10 element (Fig. 3A).

Murakami et al (65) propose a model of the RP 0 based on the RF structure, as well as the known structures of B form DNA, the α2-DNA complex and the model of the bacterial ternary elongation complex. The non-template DNA is proposed to be held in a groove between the lobe and protrusion based on cross-linking data (67). In the model, nucleotides -6 through -3 of the non-template strand are exposed (depicted in the TEC structure, Fig. 5A), consistent with previous nuclease and hydroxyl radical digestion studies that suggested this part of the non-template strand is accessible in the TEC (101). The template strand of the DNA is inserted into the active site in the RP 0, in order to base pair with initiating NTPs. To reach the active site, the template strand passes through a tunnel that is completely enclosed by σ regions 2 and 3, the β’ lid and rudder and β protrusion. The entrance to this tunnel is lined with highly conserved basic residues from σ region 2.4 and 3.0 (Fig. 4D). This positive charge may play a role in directing the negatively charged DNA into the tunnel. Downstream of the active site, the two DNA strands re-anneal and are enclosed in the RNAP between the β’ jaw and β lobe domain in the downstream DNA channel.

Open complex formation

The isomerization of the closed complex to the open complex is a multi-step process, with at least one kinetically significant intermediate (78). Analysis of the effect of temperature on the kinetics of RP 0 formation suggests that both RNAP and the DNA undergo dramatic conformational changes during this process and led to the proposal that a kink in the DNA at the -10 hexamer forms in an intermediate to RP 0 formation (78). Substitution of the -11 template strand base with 2-amino purine results in the inability of RNAP to melt the DNA (56), suggesting that melting starts at this base in the -10 element. Aromatic residues in σ region 2.3 can be seen interacting with non-template strand bases in the RF crystal structure and may help pull the strands apart. DNA melting is blocked from proceeding upstream possibly by the interaction of Trp256 with the -12 base pair (Fig. 4B).

The kink formed in the DNA by the initial unwinding at the -10 directs the downstream DNA into the entrance of the downstream DNA channel (Fig. 4C). The interaction between the downstream DNA and the RNAP triggers further closing of the channel, bringing the DNA further down into the cleft and leading to the subsequent unwinding of the DNA (78). In support of the hypothesis that downstream DNA contacts regulate the DNA-melting step of RP 0 formation, in the T. thermophilus holoenzyme structure the β gate loop (E. coli residues 370 – 381, Fig. 4A) and σ region 1.2 narrow the downstream DNA channel and prevent entry of double, but not single stranded DNA (97). In the model of the RP 0, the gate loop would interact with the major groove of the DNA at position +1 to +3, the endpoint of DNA melting (97). A large deletion in β that removes this gate loop as well as a substantial part of the lobe results in an RNAP that cannot melt the DNA downstream of -7, but, unlike wild-type RNAP, can initiate melting upstream of -7 at low temperature (82). This suggests that the gate loop, lobe, or both hinder the entry of the DNA into the downstream DNA channel, limiting the ability of holoenzyme to melt the DNA at low temperatures. Once the DNA enters the channel (Fig. 4D), however, interaction between it and the gate loop and lobe may prevent re-winding and drive completion of DNA unwinding and RP 0 formation by trapping unwinding intermediates generated by thermal fluctuation (a type of thermal ratchet mechanism).

σ region 1.1 also plays a role in RP 0 formation. This was originally suggested by the fact that substitutions and deletions in region 1.1 have been shown to impair RP 0 formation at some promoters (100, 102). Region 1.1 is proposed to be in the downstream DNA channel in the holoenzyme (Fig. 4B) and just outside the downstream channel (Fig. 4D) in the RP 0 (60). Possibly, the role of region 1.1 is to hold the downstream
DNA channel open in the holoenzyme and RPβ to allow the DNA access to the channel (66, 97). During the transition to the RPα, region 1.1 would exchange places with the DNA to sit outside the channel (Fig. 4C). This would allow the two sides of the active-site channel to close down around the DNA, with the β′ rudder and part of the protrusion interacting across the channel through the middle of the transcription bubble sealing the DNA strands apart (65).

**The elongation complex**

*The model of the elongation complex*

The structure of yeast RNAP II TEC has been solved to 3.3Å but there is as yet no structure for a bacterial TEC (34). However, the high level of sequence and structural conservation between bacterial and eukaryotic RNAP makes it possible to infer the structure of bacterial TEC from the RNAP II TEC structure. Extensive cross-linking studies between the nucleic acids and the TEC have also allowed modeling of the prokaryotic TEC (45). The structures of free and elongating RNAP II differ mainly in the position of the clamp domain, which is closed down around the RNA:DNA hybrid. The downstream DNA enters the complex through a cleft between the β′ jaw and β lobe. The 90° turn in the template strand induced during the formation of the RPα positions the +1 DNA base in the active site for base pairing with the incoming RNA nucleotide. The 8-9 base pair RNA:DNA hybrid extends through the active-site channel (Fig. 2B), positioned in between the i and i + 1 sites (Mg2+ I), but the position of the second Mg2+ ion (Mg2+ II) is not as clear. Mg2+ II is not present in the *T. aquaticus* core crystal structure and is in two different positions in the *T. thermophilus* holoenzyme and RNAP II structures (22, 97, 109). This more weakly bound Mg2+ ion is thought to be brought into the active site by the incoming NTP. Sosunov et al. (90) have recently proposed an alternate Mg2+ binding site based on modeling and substitutions made in the active center (Fig. 5B). In their model, Mg2+ II is coordinated by two of the three aspartate residues of the NADFDGD motif and stabilized by the phosphate of the incoming NTP. Unlike the positions assigned Mg2+ II in the crystal structures, this position fits the requirements for the S1,2 geometry of the phosphodiester bond formation chemistry, as well as the nuclease and pyrophosphorylase activities of the active center (90). NTP gains access to the active center through the secondary channel. A binding site for the incoming NTP at the entrance to the secondary channel ("E site," Fig. 5B and C) has been proposed based on modeling and mutagenesis of the active site (90); NTP bound at this site has been detected in crystal structures (70).

The presence of alternate conformations of the bridge helix and trigger loop in the active site in the different crystal structures has led to conjecture into the possible mechanism of catalysis (Fig. 5B and C). In the RNAP II TEC structure, the bridge helix is straight and the 3′ end of the nascent RNA lies next to the helix in the i + 1 site (34). However, in the bacterial RNAP structures, the bridge helix is bent or unfolded near the active site, with side chains suggesting this domain holds the hybrid in the active-site channel. The active-site channel forms a complementary pocket for binding the hybrid, but the specificity is for the phosphate backbone, not specific bases. Several of the residues that contact the backbone interact with two phosphate groups simultaneously, possibly reducing the activation energy required for translocation. Also, the binding pocket is lined with positive residues that may attract the hybrid sequence-non-specifically (34). The single-stranded RNA transcript in the RNA exit channel has also been shown to be important for the stability of the elongation complex (46, 103), possibly due to the flap domain closing down around the RNA. Together, these contacts allow for tight but sequence-non-specific binding to the DNA template.

*The mechanism of catalysis*

The RNAP crystal structures reveal the architecture of the active center (Fig. 5B). The universally conserved NADFDGD motif of the β′ subunit chelates a Mg2+ ion deep in the active site channel (Fig. 2B), positioned in between the i and i + 1 sites (Mg2+ I), but the position of the second Mg2+ ion (Mg2+ II) is not as clear. Mg2+ II is not present in the *T. aquaticus* core crystal structure and is in two different positions in the *T. thermophilus* holoenzyme and RNAP II structures (22, 97, 109). This more weakly bound Mg2+ ion is thought to be brought into the active site by the incoming NTP. Sosunov et al. (90) have recently proposed an alternate Mg2+ binding site based on modeling and substitutions made in the active center (Fig. 5B). In their model, Mg2+ II is coordinated by two of the three aspartate residues of the NADFDGD motif and stabilized by the phosphate of the incoming NTP. Unlike the positions assigned Mg2+ II in the crystal structures, this position fits the requirements for the S1,2 geometry of the phosphodiester bond formation chemistry, as well as the nuclease and pyrophosphorylase activities of the active center (90). NTP gains access to the active center through the secondary channel. A binding site for the incoming NTP at the entrance to the secondary channel ("E site," Fig. 5B and C) has been proposed based on modeling and mutagenesis of the active site (90); NTP bound at this site has been detected in crystal structures (70).

Fig. 5. Structure of the TEC (A) A TEC model based on the core RNAP model shown in Fig. 2 with mobile modules (23) adjusted to the conformation of the *S. cerevisiae* RNA II TEC (PDB ID 1I6H; reference 34). The RNA:DNA hybrid and downstream DNA positions are those observed in the *S. cerevisiae* RNAP II TEC (34) with the scaffold dimensions and upstream DNA as modeled by Korzheva et al. (45). Subunits and DNA are colored as in Fig. 2. RNA is red. Active site Mg2+ ions are yellow. The trigger loop is depicted as an orange worm. The β D loop II is shown as a dark blue worm. The box encloses the portion of the active-site channel magnified in B and C. (B) The conformation of the active site in *T. thermophilus* holoenzyme (97). A portion of the non-template strand of the DNA has been removed to allow a clearer view of the active center. Ovals represent the i, i + 1 and E sites. The RNA 3′ end is depicted in the i + 1 site, however, the kinked bridge helix in this structure would sterically clash with the base in the i + 1 site. (C) The conformation of the active site in the yeast RNAP II TEC (34). The view is the same as in B. In this conformation, the bridge helix is straight and there is no steric clash between the bases in the i + 1 site and residues from the helix.
The active site of RNAP is responsible not only for nucleotide addition, but also for other functions of the active site. The active-site gate may be analogous to the O-helix in DNA polymerases; this movement of which facilitates proper alignment of the $i + 1$ and $i$ sites and allows PPi release (27); a similar conformational change is proposed to drive an active site rearrangement (28) requires for catalysis. In DNA polymerases, accumulation of negative charge on the newly formed pyrophosphate is proposed to drive active site rearrangement that translocates the 3' nt from the $i + 1$ to the $i$ site and allows PPi release (27); a similar scenario may occur in RNAP.

Other functions of the active site
The active site of RNAP is responsible not only for nucleotide addition, but also for discriminating ribo- from deoxyribonucleotides, maintaining the fidelity of transcription and in some cases cleaving the RNA transcript, either as a means of proofreading or to escape from a backtracked state. The conformation of the $i + 1$ site may be involved in maintaining transcriptional fidelity. A rif $R$ substitution in the β subunit that increased misincorporation (ack-1) maps between residues 565 and 576, which includes part of the β D loop II (Fig. 5B) that lies over the $i + 1$ site (33). Possibly, altering the structure of this loop allows non-Watson-Crick base pairs to fit better into the active site, increasing the rate of addition of mismatched NTPs. Cleavage of the nascent transcript is also involved in maintaining fidelity (31). The active-site channel is complementary to the conformation of the RNA:DNA hybrid, but not to the conformation of double-stranded DNA. Misincorporation of dNTPs, as well as mismatched nTPs, into the nascent RNA would distort the hybrid and possibly decrease the stability of the complex, causing the RNAP to backtrack along the RNA and DNA chains to a point where correctly synthesized RNA would be in the hybrid and the misincorporated nt would be in the secondary channel (34). This complex would then be subject to the action of the Gre A/B cleavage factors, which bind in the outer entrance of the secondary channel (73) with a coiled-coil domain in its amino-terminus extending through the channel up to the active site (70), and stimulate the intrinsic nuclease activity of the active site (9). Highly conserved acidic residues in the Gre factors’ coiled-coil domain may modify the active site to catalyze the cleavage reaction, either by directly stabilizing a Mg$^{2+}$ II ion in the position proposed by Sosunov et al or disrupting a salt bridge between an aspartate residue at β 814 and β R1106, allowing β D814 to chelate the second Mg$^{2+}$ ion (70, 90).

Initiation of transcription and promoter escape
Formation of the first phosphodiester bond RNAP initiates transcription de novo from two NTPs, not from a primer like the DNA polymerases. This means that the first phosphodiester bond forms between two nucleotide triphosphates in the $i$ and $i + 1$ sites; each NTP would bind a Mg$^{2+}$ ion. Therefore, during transcription initiation, the active center must accommodate three Mg$^{2+}$ ions. The γ phosphate of the initiating NTP cross-links to a section of the α loop 3.2 (83) that protrudes into the active site (66, 97). Possibly, this loop helps to chelate the Mg$^{2+}$ ion associated with the NTP in the $i$ site through highly-conserved acidic residues, one of which is located ~15 Å from the γ phosphate of the initiating NTP. A truncated α lacking region 3.2 forms a holoenzyme with a lowered affinity for the initiating NTP (19). Once this first phosphodiester bond is formed, translocation occurs and NTP binding is no longer required in the $i$ site.

Abortive initiation
The initiation of transcription is characterized by a competition between transcript elongation and release. Successful elongation requires that RNAP disengage from contacts with the promoter sequences and begin translocating down the template. Before this happens, RNAP goes through several rounds of abortive transcription in which a short RNA product is synthesized and released, while RNAP remains at the promoter (98). In the holoenzyme structure, the α region 3.2 loop occupies the active-site channel and the RNA-exit channel (Fig. 4D), blocking extension of the RNA product past a few nucleotides (66). This suggests that the competition between transcript elongation and release reflects the competition between the 3.2 loop and the RNA to fill the active-site channel and RNA-exit channel. A holoenzyme formed with a α$^{+}$ subunit truncated before region 3.2 exhibits decreased abortive initiation, supporting the competition model (66). Once the transcript reaches approximately 12 nt, it will have
displaced the 3.2 loop (Fig. 6A). This may disrupt the interaction between σ region 4 and the flap and result in destabilizing the interaction between σ and the -35 element of the promoter, initiating the process of promoter escape (Fig. 6B) and the transition into the elongation complex (Fig. 6C). The rate at which the initiating RNAP escapes from the promoter and the species of abortive products generated are both influenced by the sequences in the promoter (98).

σ release

The release of σ from the elongating complex is a multi-step process. In the open complex, σ is tightly bound to core, with contacts between σ region 2.2 and the β′ coiled coil, σ region 3.2 and the RNA exit channel, and σ region 4 and the flap domain (66, 97). During the process of abortive initiation, region 3.2 is displaced from the RNA exit channel. This, in turn, may disrupt the interaction of σ region 4 with the flap. In fact, at the σ-dependent promoter-proximal pause site in λ P10′, σ region 4 has been repositioned in the complex by the anti-terminator Q such that it has left the flap and is bound to a -35 like sequence located immediately upstream from the pause site, arguing that as the polymerase leaves the promoter the interaction between core and at least parts of σ is weakened (59, 68). Once the contacts with σ region 3 and 4 are lost, the residual region 2 interaction is lost slowly and stochastically (87). Stochastic release of σ weakly bound to the TEC may explain why some studies have detected σ persisting in the elongation complex (4, 63) even though experiments with reconstituted elongation complexes establish that RNA and σ compete for binding to RNAP (24). Furthermore, estimates of the in vivo activity of free σ suggest it may be capable of rebinding the TEC during the course of transcript elongation (110).

Conclusions

The publication of crystal structures for multiple forms of RNAP has made possible a much more detailed examination of the function of the enzyme and the mechanisms of catalysis, promoter recognition and transcriptional activation. However, many questions remain to be answered. What is the structure of σ region 1.1 and where is it located in free σ, the holoenzyme and RP? What is the mechanism of strand separation during open complex formation? How is the first phosphodiester bond formed? What are the conformational changes in the active site and what are their roles in catalysis and translocation? What features of the active site are required to maintain transcriptional fidelity? How do pause sites dramatically slow the rate of nucleotide addition, and how do termination signals dissociate the TEC? Many of these questions can be addressed with additional crystal structures. For example, solving the structure of the TEC with a non-hydrlysable nucleotide analog in the i + j site would be informative by showing the conformation of the active site immediately before nucleotide addition. Solving the first RNAP crystal structures is only the beginning of the road to understanding the function of the enzyme.

References


72. Pati, U. K. 1994. Human RNA polymerase II subunit hRPB14 is homologous to yeast RNA polymerase I, II, and III subunits (ACG1, 2G05, and YBB1) and is similar to a portion of the bacterial RNA polymerase alpha subunit. Gene 145:289-92.


