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New Concepts

Topological and Conformational Analysis of the Initiation and Elongation Complex of T7 RNA Polymerase Suggests a New Twist

Karsten Theis,*,‡ Peng Gong,§ and Craig T. Martin§

Department of Biochemistry & Molecular Biology and Department of Chemistry, University of Massachusetts Amherst, Amherst, Massachusetts 01003

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ABSTRACT: The N-terminal domain of T7 RNA polymerase undergoes large conformational changes in the transition from transcription initiation to elongation. The rigid body displacement of parts of the N-terminal domain (residues 72−152 and 204−258) has been described as a screw motion composed of a rotation by 140° and a translation of >20 Å along the rotation axis. Protein−protein interactions between residues 23−42 and the C-terminal domain are present in both the initiation and the elongation complex. Assuming that these interactions are retained during the transition between the two states, we find that topological constraints require a right-handed 220° screw motion of the N-terminal rigid body rather than the proposed 140° left-handed screw motion. In the initiation complex, a loop (residues 153−203) extruding from the N-terminal rigid domain wraps around the N-terminal 30 residues. Assuming the N-terminal rigid domain stays folded during the transition, the N-terminus has to pass through this loop before the rigid domain can undergo the translation leading to the elongation complex. On the basis of these topological constraints, we suggest an alternate sequence of conformational changes leading from transcription initiation to elongation in T7 polymerase.

The past several years have seen a wealth of crystal structures of RNA polymerases, both single subunit and multisubunit (*1*−*7*). From these structures, bacterial and eukaryotic RNA polymerases are now known to share a common structural ancestor, while the single subunit RNA polymerase from bacteriophage T7 appears to be related to the Pol I family of DNA polymerases. Despite the lack of structural similarity, the fundamental mechanisms of transcription appear to be the same for single subunit and multisubunit RNA polymerases. They all show promoter-dependent binding and melting of their target DNAs, followed by the transition to an initially unstable, abortively cycling transcribing complex. In all systems, this complex converts to the more traditional, stable elongation complex after the synthesis of about 10 nucleotides of RNA. Similarly, in all systems, the final heteroduplex is about 8 base pairs in length. Finally, all RNA polymerases recognize a classic stem hairpin (ρ-independent) terminator. The small size and relative simplicity of both enzyme and promoter make the single subunit T7 system an ideal model in which to study fundamental aspects of transcription.

Crystal structures for a promoter bound complex with a three base transcript and for a model of an elongation complex reveal a large-scale structural change occurring in the complex (*1*−*3*). Thus, the initially transcribing, highly abortive phase of transcription is accompanied by a large

* To whom correspondence should be addressed. E-mail: theis@biochem.umass.edu. Tel: +1 413 577 2890. Fax: +1 413 545 3291.
‡ Department of Biochemistry & Molecular Biology.
§ Department of Chemistry.

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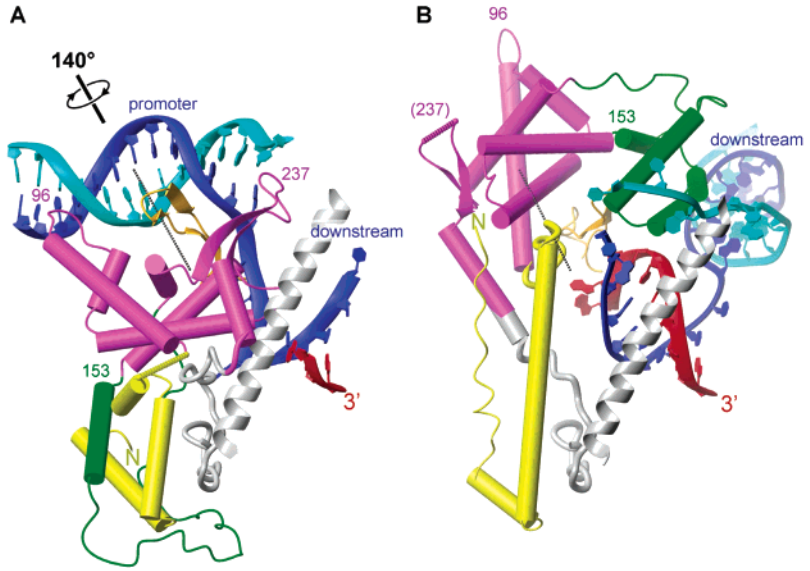

FIGURE 1: Structural changes of the N-terminal part of T7 RNA polymerase in the transition from initiation (left, PDB code 1QLN (*1*)) to elongation (right, PDB code 1MSW (*2*)). In the transition, parts of the N-terminal domain of T7 RNA polymerase undergo a rigid body motion (rigid domain, residues 72−152 and 204−258, shown in magenta). The black dotted line indicates the axis of the rigid body rotation; its length indicates the magnitude of the translation along the axis. The clockwise direction of rotation (with respect to a top view) suggested previously (*2*, *3*) is indicated on top of panel A. To orient the two complexes, the C-terminal domains (residues 267−883) were superimposed, but largely omitted from the figure for clarity. Yellow: N-terminal residues through 71. Green: refolding loop (residues 153−203). Gray: selected residues of the C-terminal domain (residues 259−285 as worm, residues 378−409 of the thumb domain as ribbon). Orange: specificity loop of the C-terminal domain (residues 738−769). Blue and cyan: template and nontemplate DNA strands. Red: RNA transcript. Residues 56−71 in the initiation complex and residues 233−240 in the elongation complex are disordered (dotted connections). Selected residues are labeled. This Figure and Figures 3, 4, and S1−4 were prepared using molmol (*16*).

change in the structure of the complex. In particular, a large rigid body rotation (and translation) of the bulk of the N-terminal domain (Figure 1, magenta) makes way for the growing heteroduplex and simultaneously both serves to disrupt promoter binding and allows refolding of a loop (Figure 1, green) to generate an RNA exit channel.

Two fundamental questions remain unanswered. First, what is the timing of the structural changes with respect to translocation, and second, what are the intermediate conformations leading to abortive cycling, template release, or highly processive elongation? Steric clashes predicted from the modeling of hybrid growth argue for some change in conformation as early as translocation to position +4. The protein structure in the complex with a 3 base transcript is identical to that of an uninitiated complex (*1*, *8*, *9*), so the transition starts at some point after the first 3 bases are transcribed. Does the rigid body motion of the N-terminal rigid domain proceed in a stepwise fashion with the incremental growth of the hybrid, or do subtle changes first lead to a strained conformation which then proceeds to a rapid rearrangement in a large, concerted motion? Both Yin et al. (*2*) and Tahirov et al. (*3*) describe the movement of the N-terminal rigid domain in the transition from initiation to elongation as a 140° left-handed screw rotation (i.e. a clockwise direction of rotation with respect to a top view of Figure 1). However, our analysis of the connectivity reveals topological constraints suggesting instead a 220° right-handed screw rotation. Moreover, analysis of the disrupted and newly formed interfaces leads to a proposal in which the initiation complex is distorted to make room for the growing heteroduplex by detaching the N-terminal domain from the C-terminal domain with only minimal rotation and full retention of promoter−polymerase interactions. Only when the accumulated conformational strain caused by this movement exceeds the promoter binding energy is the loss of the bound promoter DNA and the rotation of the N-terminal rigid domain expected to occur.

What Is the Overall Motion of the N-Terminal Rigid Domain? To analyze the conformational differences between the crystal structure of the initiation complex (*1*) and the elongation complex (*2*, *3*) of T7 RNA polymerase, we superimposed the molecules and measured the distance between corresponding atoms in the 263 N-terminal residues (Figure 2A). On the basis of superposition of the N-terminal domains (Figure 2A, + symbols), we define here the N-terminal rigid domain as residues 72−152 and 204−258, which move as a rigid body by more than 20 Å between the initiation and the elongation complex, and the refolding loop as residues 153−203, which change their backbone conformations to form a new hydrophobic core in the transition. Superposition of the C-terminal domains of the initiation and elongation complexes (Figure 2A, filled symbols) shows that the N-terminal rigid domain and the refolding loop are displaced by more than 20 Å relative to the C-terminal domain, as described previously (*2*, *3*), but a stretch of 20 residues (residues 23−42) shows little change in its position. In fact, this 20 amino acid stretch is the only segment of the N-terminal domain that retains both its original conformation and its contacts with the C-terminal domain from initiation to elongation.

If all contacts of the N-terminal domain with the C-terminal domain in the initiation complex were lost before the new contacts observed in the elongation complex were formed, the N-terminus would be constrained only by its covalent attachment to the C-terminus and might sample many nonproductive orientations in the transition between the two states. An alternative possibility is that the interactions between residues 23−42 and the C-terminal domain

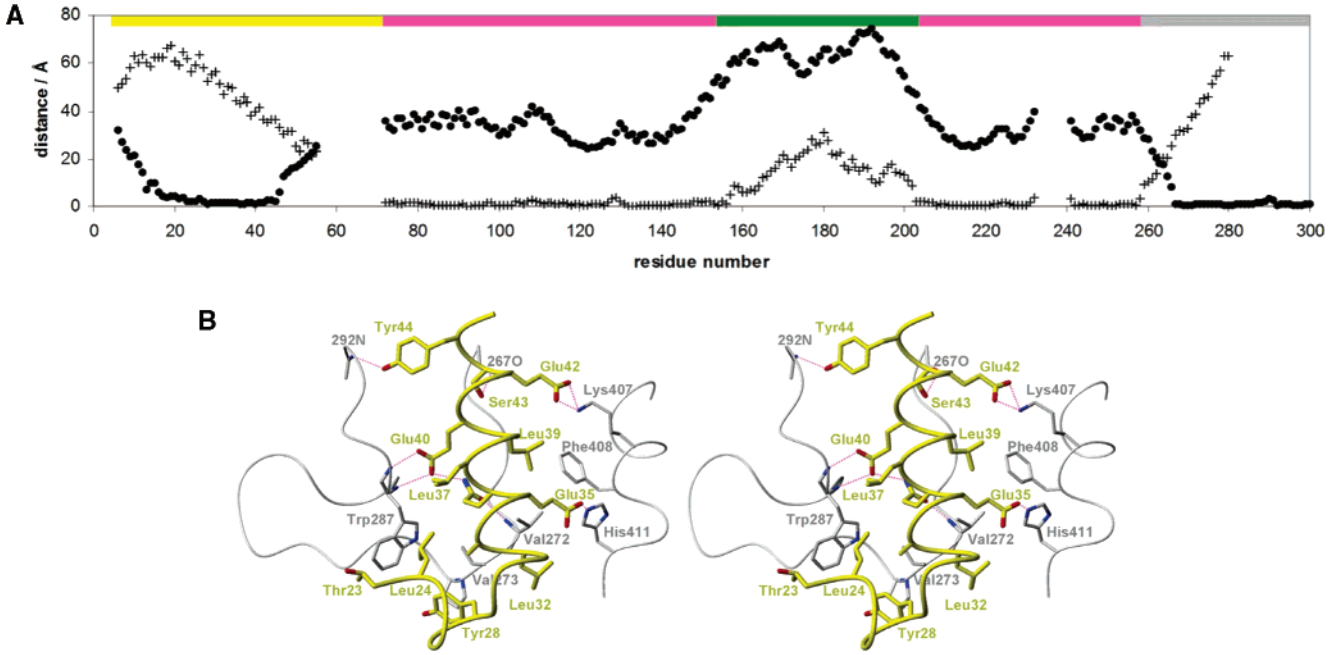

FIGURE 2: Rigid domains in the transition from transcription initiation to elongation. (A) The distance between corresponding Cα atoms in the N-terminal 300 residues of the initiation complex (PDB code 1QLN) and the elongation complex (PDB code 1MSW) is shown after superposition of the C-terminal domains (residues 267−883, filled circles) and after superposition of the N-terminal rigid domains (residues 72−152 and 204−258, + symbols). The superpositions are shown in Figures 1 and 4, respectively. Domains are indicated by the horizontal bars using the color scheme of Figure 1. (B) Stereodiagram of the interface between residues 23−42 (yellow) and the C-terminal domain (gray) of the elongation complex (PDB code 1MSW). View from the left with respect to Figure 1. Selected side chains of the interface are shown and labeled. The hydrogen network conserved from transcription initiation (structure 1QLN) to elongation is shown as dashed magenta lines. Ser 43 and Tyr 44 show conserved hydrogen bonds with the main chain of the C-terminal domain and are included in the figure.

are retained during the transition, thus tethering both the N-terminal and C-terminal ends of the moving N-terminal domain.

The interactions of residues 23−42 with the C-terminal domain are substantial, occluding more than 500 Å^2 of the surface area of these residues in the interface. Residues Thr 23, Leu 24, Tyr 28, Leu 32, and Leu 37 form a hydrophobic patch that contacts Val 273, Pro 274, and Trp 287 (Figure 2B). A smaller cluster of hydrophobic residues comprises residues Leu 39, Val 272, and Phe 408. In addition, the hydrogen bonding network observed in the interface is similar in the initiation and elongation complexes (Figure 2B shows the hydrogen bonds observed in both 1QLN and 1MSW). Observed differences in side chain conformations and hydrogen bonding might be due either to limited accuracy of the structures or to subtle changes in the interface before and after the transition. Overall, however, the interface is well conserved. In the following, we explore the consequences of retaining this interface while the N-terminal rigid domain undergoes the transition from initiation to elongation.

Retaining interactions between residues 23−42 and the C-terminal domain results in two rigid bodies, the N-terminal rigid domain on one hand (symbolized by the magenta wedge in Figure 3A) and residues 23−42 and the C-terminal domain on the other (symbolized by the yellow and gray blocks), tethered by two connections. Rotating the top parts of the structure counterclockwise (with respect to a top view) against the bottom parts leads to a right-handed twist (Figure 3A, top). Conversely, a clockwise rotation leads to a left-handed twist (Figure 3A, bottom), with the N-terminal connection passing under the C-terminal one. If the N-terminal end were free to move around the C-terminal end, a transition between the two twisted structures would be possible without rotating the top part of the structure. If both ends are immobile, however, we can infer the sense of rotation during the transition from the handedness of the twist in the product structure. Inspection of the crystal structures of the initiation and elongation complex reveals that the N-terminal and C-terminal connections of the N-terminal rigid domain are not twisted in the initiation complex[1] (compare Figure 3, panel B left and panel A left) and have a right-handed twist in the elongation complex (compare panel B right and panel A top right). Thus, if the N-terminal residues 23−42 remain attached to the C-terminal domain, the N-terminal rigid domain must undergo a right-handed screw motion in the transition from initiation to elongation.

Refolding of Residues 153−203. In addition to the rigid-body movement of large parts of the N-terminal domain analyzed above, the transition between transcription initiation and elongation involves refolding of a loop (the refolding loop) extruding from the N-terminal rigid domain. In the initiation complex, the refolding loop wraps around the N-terminal 30 residues, whereas in the elongation complex, it has a compact structure and the N-terminus is free (Figure 4). Assuming that the N-terminal rigid domain stays folded during the transition, the two conformations are topologically

[1] Because residues 56−71 of the N-terminal connection are disordered in the initiation structure, the path of this stretch of polypeptide is not experimentally determined. The disorder, however, suggests that these residues do not interact with the protein but rather point out into the solvent. In particular, it should be noted that there is no space for these residues to wrap around the C-terminal connection in the initiation complex structure. This means that the disorder of residues 56−71 does not affect our analysis.

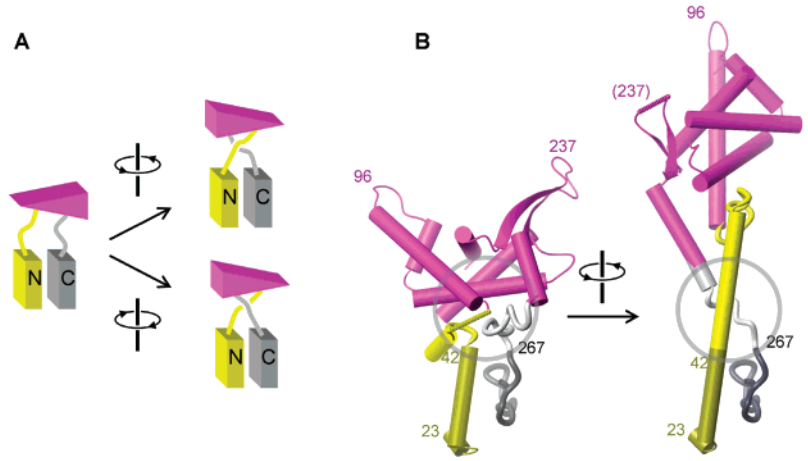

FIGURE 3: The direction of rotation of the N-terminal rigid body domain. (A) Rotating an object (wedge, magenta) that is tethered by two flexible connections to a fixed support (blocks labeled N and C) in two different directions yields distinct twists of the connections. (B) The N-terminal rigid body domain (magenta) and its N-terminal and C-terminal connections in the initiation (left) and elongation (right) conformation. The N-terminal residues 23−42 (shaded yellow) and C-terminal residues 267 to 285 (dark gray), which show conserved conformations and interactions from initiation to elongation (and are symbolized by blocks in panel A), connect to the rigid domain (magenta, symbolized by the wedge in panel A) via two tethers (residues 43−71 and 259−266 shown in yellow and gray, respectively). The tethers are emphasized by the large open circles, and the proposed counterclockwise direction of rotation of the rigid domain is indicated. Residues 56−71 are disordered in the initiation complex structure. The molecules are oriented as in Figure 1, and residues 153−203 (refolding loop) have been omitted for clarity.

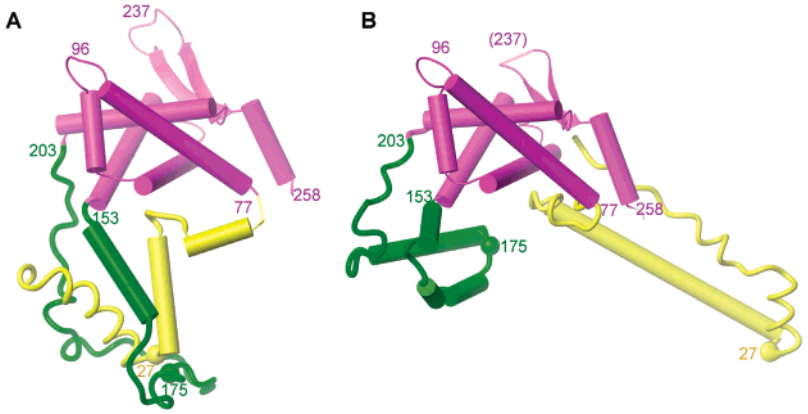

FIGURE 4: The refolding loop (green) wraps around the N-terminus (yellow) in the initiation complex (left, PDB code 1QLN), but not in the elongation complex (right, PDB code 1MSW). Selected residues are labeled. The distance between the Cα atoms of residues 27 and 175 (shown as yellow and green sphere, respectively) increases from 5 to 52 Å, illustrating the extent of the structural rearrangement. Orientation: left is rotated approximately 60° about a vertical axis with respect to Figure 1 left. Right is shown such that N-terminal rigid domain has the same orientation in the two panels.

distinct, implying that the N-terminus has to thread through the refolding loop in the transition. While wrapped around the N-terminus, the refolding loop restrains movements of the N-terminal rigid domain. Specifically, the N-terminal rigid domain can only translate upward (with respect to the orientation in Figure 1) and rotate to its position observed in the elongation complex after the refolding loop and the N-terminus are disentangled, suggesting that the refolding loop has to disengage from the N-terminus at an early stage in the transition.

In the initiation complex, a large portion of the refolding loop contacts the remainder of the polymerase (Figure 5, black bars). Interactions between the refolding loop and residues 7−45 of the N-terminal domain amount to an interface area of 1050 Å^2, and interactions with residues 274−305 of the C-terminal domain to an interface area of 600 Å^2. In the elongation complex, the refolded loop forms a hydrophobic core of its own; the total surface area of the loop decreases by about 30% or 1900 Å^2 on refolding, burying residues Ile 154, Leu 157, Phe 162, Val 166, Leu 170, Phe 182, Met 183, and Val 186 in its hydrophobic core. The only substantial interactions of the refolded loop with the C-terminal domain are mediated by Met 750 and Phe 751, which insert into a pocket of the newly formed hydrophobic core.

In refolding, the loop undergoes a conformational change involving movements of more than 20 Å (with respect to the N-terminal rigid domain, see Figure 4). These large movements are possible neither in the initiation nor in the elongation conformation because they would result in steric clashes with the N-terminus and the DNA/RNA hybrid, respectively (Figure 1). To refold the loop, the complex has to go through intermediate conformations that prevent these steric clashes. The right-handed screw rotation of the N-terminal rigid domain proposed here suggests the nature of such intermediates. (It should be noted that the proposed counterclockwise rotation requires an angle of rotation of 220° compared to 140° for the opposite direction, and that

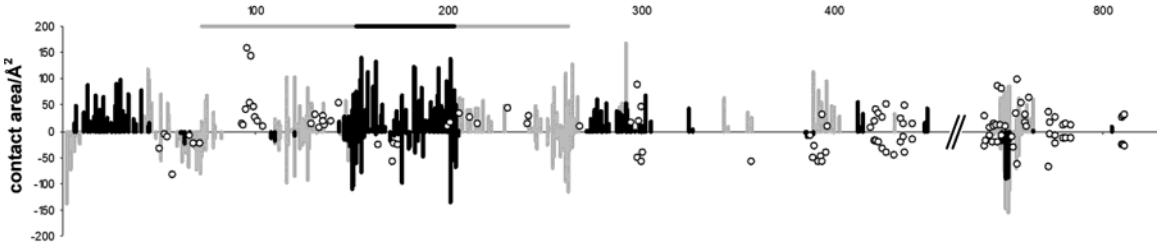

FIGURE 5: Changes in contact area during the transition from initiation to elongation observed in structures 1QLN and 1MSW on a by-residue basis calculated with molmol (*16*). The contact area between protein and nucleic acids (open circles) was computed as the difference in accessible surface area of the protein residues before and after removing nucleic acid atoms from the coordinates. Solid vertical bars show the surface area buried in the interface between the refolding loop (residues 153−203) and the remainder of the protein, the gray vertical bars the surface area buried between the N-terminal rigid domain plus the refolding loop (residues 72−263) and the remainder of the protein. Protein−protein contact areas were calculated in the absence of nucleic acid coordinates as the difference of accessible area calculated for the separated and combined parts of the protein. For all calculations, positive values refer to the initiation complex and negative values to the elongation complex. Residues 460−720 and 820−883, which do not contact the N-terminal domain and show no change in nucleic acid binding surfaces, were omitted from the graph.

the intermediates visited along the two paths are distinct.) First, the rotation would move the refolding loop away from the remainder of the protein into the solvent, where it could form its own hydrophobic core unperturbed by possible competing interactions with the remainder of the protein. Near completion of the 220° rotation, the refolded loop would reapproach the C-terminal domain, poised to bind to residues Met 750 and Phe 751. These interactions are crucial because they complete the tunnel through which the RNA exits the active site, ensuring high processivity of the elongating enzyme (*2*, *3*).

The Extent of Conformational Change Possible without Promoter Release. The active site of T7 RNA polymerase in its initiation conformation provides sufficient space for an RNA/DNA hybrid of 3−4 base pairs (*1*). Modeling longer hybrids into the initiation complex results in steric clashes with the N-terminal rigid domain, suggesting that this domain has to move away from the active site as the hybrid grows. On the other hand, cross-linking studies suggest that the −17 promoter contacts are retained but suggest that −9 and −5 contacts are disturbed on translocation to position +7 (*10*). Footprinting data show retention of promoter contacts on translocation through at least position +6 (*11*) or even +8 (*12*). Fluorescence data show that not only is the initial bubble fully melted in complexes stalled at position +8 but the eight base pair heteroduplex is intact as well (*13*). Chemical nuclease data show that promoter binding elements stay in proximity to the promoter through position +7 (*14*). More recent studies have led to a model in which collapse of the upstream edge of the initially melted bubble contributes to both displacement of the RNA and the loss of promoter contacts (*15*). Finally, biochemical data agree that after transcribing up to 8 nucleotides, the promoter is still bound and the polymerase can revert to the initiation state in the event of transcript loss. Therefore, an intermediate conformation must exist that supports promoter binding and provides space for the growing hybrid at the same time.

In the crystal structure of the initiation complex, the N-terminal rigid domain has extensive interactions with the remainder of the polymerase and with the promoter DNA. Apart from the N- and C-terminal covalent tethers discussed above, the N-terminal rigid domain binds noncovalently to the specificity loop and to the thumb of the C-terminal domain (Figure 5, gray lines), as well as to the promoter DNA and the DNA/RNA hybrid (Figure 5, open circles). In a modeling exercise, we explored which of these interactions must be disrupted when moving the N-terminal rigid domain away from the active site to provide room for the growing hybrid. The promoter DNA can remain bound if it moves away from the active site together with the N-terminal rigid domain. This is feasible because the number of nucleotides in the template between the promoter region and the nucleotide in the active site pocket increases as the RNA polymerase transcribes. Many of the contacts between the N-terminal rigid domain and the C-terminal domain will be disrupted, for example those between the N-terminal rigid domain and residues 398−403 of the thumb region of the C-terminal domain (Figure 5). However, the specificity loop contacting the N-terminal rigid domain (and the promoter) can move to a degree limited by the length and flexibility of the loop by which it is tethered to the C-terminal domain (*3*). By crude modeling, we estimate that the loops connecting the specificity loop to the bulk of the C-terminal domain allow a movement of the specificity loop of up to 10 Å when fully extended, while further movement would disrupt the secondary structure of the C-terminal domain. Thus, relative to the structure of the initiation complex with a three nucleotide RNA product (*1*), the N-terminal rigid domain can move away from the C-terminal domain by about 10 Å—corresponding to about 3 additional base pairs—without disrupting the binding interface between T7 RNA polymerase and the promoter. A concerted translation of the N-terminal rigid domain, the specificity loop of the C-terminal domain, and the promoter DNA away from the core C-terminal domain would therefore allow transcription to proceed to at least position +6 before additional conformational changes must occur.

Sequence of Events in the Transition. In this analysis, we have identified constraints on the intermediates between transcription initiation and elongation that suggest a sequence of events in the transition. In our model, we divide the transition into three steps.

(1) The N-terminal rigid domain detaches from the C-terminal domain without releasing the promoter, and the refolding loop disentangles from the N-terminus.

(2) The N-terminal rigid domain loses its contacts with the specificity loop and the promoter.

(3) The N-terminal rigid domain undergoes a counterclockwise rotation by 220° to its position observed in the elongation complex.

These steps are sequential because disrupting interactions between the refolding loop and the N-terminus in step 1 enables the conformational changes of steps 2 and 3, and disrupting interactions between the N-terminal rigid domain and the specificity loop in step 2 enables the conformational changes of step 3. To illustrate the proposed sequence of events and explore the consequences and possible problems in the model, we have assembled a series of conformations showing the envisioned stages of the transition into a movie (Supporting Information Figures S1, S2, and S3).

As the first step in our model, the N-terminal rigid domain moves away from the active site, making room for the growing heteroduplex as described above (Figure S1). Because the refolding loop extrudes from the N-terminal rigid domain, movement of the rigid domain would help in "peeling" the refolding loop away from the N-terminal 20 residues, a prerequisite for larger movements of the N-terminal rigid domain. In the crystal structure of T7 RNA polymerase complexed with lysozyme (PDB code 1ARO (*9*)), residues 165−181 of the refolding loop are disordered, indicating flexibility in solution and a possible starting point for refolding (in the initiation complex structure, PDB code 1QLN, this part of the refolding loop engages in crystal packing contacts). On the other hand, residues 195−201 are ordered in both structures, with Leu 195 and Trp 204 buried in hydrophobic regions, blocking the RNA exit path observed in the structure of the elongation complex. In the lysozyme-inhibited structure, these residues are in proximity to the bound lysozyme. One could imagine that binding of lysozyme interferes with refolding of the loop by steric hindrance, which would explain the observed increase in abortive cycling in the presence of lysozyme (*9*). This first step in our model of the transition, movement of the N-terminal rigid domain away from the active site, ends when the specificity loop is stretched to its limit and its interactions with the promoter and the N-terminal domain are disrupted. Alternatively, the newly synthesized RNA is released during this step, and the conformational changes reverse, leading to abortive cycling without release of the promoter DNA.

In the second step of our model, promoter clearance, the promoter dissociates from the C-terminal specificity loop and the binding interface of the N-terminal rigid domain (Figure S2). This is in contrast to the idea that the N-terminal rigid domain might remain bound to the promoter throughout the transition to the elongation conformation, as proposed by Tahirov et al. (*3*). In our model, the N-terminal rigid domain undergoes a larger rotation (220° counterclockwise instead of 140° clockwise), precluding the possibility of the specificity loop "slipping out" of the promoter binding interface while the promoter remains bound to the N-terminal rigid domain. In the crystal structure of the initiation complex, the N-terminal rigid domain contacts the promoter DNA (see Figure 5). The so-called intercalating hairpin with Val 237 at its tip is located at the upstream end of the transcription bubble, preventing the strands from reannealing. This hairpin has to move out of the way before the bubble can collapse, but in the initiation complex, its movement is restrained by the proximity of the C-terminal thumb domain. However, if the N-terminal rigid domain moves away from the C-terminal domain as suggested in our model, the hairpin loop would have enough space to move away from the promoter DNA. Chemical nuclease data of T7 RNA polymerase conjugated to iron at residue 239 are consistent with the idea of increased flexibility of the intercalating hairpin during the transition; as transcription proceeds from position +1 to +7, the number of consecutive sites on the DNA template cleaved increased from two to six (*14*). In the elongation complex, the intercalating hairpin is disordered, suggesting that its conformation is flexible enough to let the template strand (and the specificity loop) pass it in preparation for bubble collapse.

Disentangling the refolding loop from the N-terminus followed by promoter release sets the stage for the right-handed rotation of the N-terminal rigid domain, which is the third step in our model (Figure S3). At some time during this rotation, the refolding loop forms its hydrophobic core. In addition, the N-terminal and C-terminal connections of the rigid domain (residues 50−71 and 257−267) become largely α-helical, with two helices of the N-terminal connection merging to a single helix encompassing residues 29−60, and the helix 247−257 preceding the C-terminal connection extending to residue 262. With the formation of these α-helices, large parts of the exit tunnels for the RNA and the template DNA are formed. The upstream end of the DNA bubble, no longer held open by the intercalating loop and tethered via the promoter, is able to collapse, and the reannealed duplex can move (down and toward the viewer in the orientation of Figure 1) to occupy the space in which it is observed in the elongation complex, but which is occupied by the N-terminal rigid domain prior to the transition. (The DNA and the N-terminal rigid domain effectively trade places in the transition, which means that only one of these structures can move into place directly, while the other has to move around the incoming player.) Because the protein structure is quite open at this stage in our model, the nontemplate strand can help to peel the template strand off the 5′ end of the RNA, which then can exit the polymerase active site and move away from the template strand upon further elongation of the transcript.

As mentioned above, the right-handed screw motion of the N-terminal rigid domain leads to intermediates in which the refolding loop points away from the remainder of the complex, allowing it to assume its new compact conformation. While the order of the events after promoter release is difficult to predict (indeed, some motions may be independent of others and occur stochastically), formation of the interactions between the refolded loop and the specificity loop, which changes its secondary structure in the transition, is expected to occur as the final step in the transition. This traps the RNA leaving the active site in an exit tunnel, completing the transition to the processive elongation complex.

Large-scale protein conformational change is crucial for many biological events, but its mechanism is poorly understood. T7 RNA polymerase is a molecular machine whose conformational states are particularly well-studied, but the path connecting these states is unknown. On the basis of a topological and conformational analysis, we suggest a three-step sequence of conformational changes leading from transcription initiation to elongation. In contrast to previous suggestions, our model features an overall right-handed 220° screw motion of the N-terminal rigid domain, providing a mechanism for the changing roles of this domain from promoter binding element to processivity factor. The model

is a testable hypothesis because it specifies the order in which interactions are disrupted and newly formed.

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SUPPORTING INFORMATION AVAILABLE

Figures S1−3: The proposed sequence of conformational changes in three steps. Figure S4: The entire sequence showing protein Cα atoms, only (as spheres with 3 Å radius). Color scheme as in Figure 1. Figures S2, S3, and S4 are a top view with respect to Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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