

Two Conformations of RNA Polymerase II Revealed by Electron Crystallography

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A new two-dimensional crystal form of yeast RNA polymerase II was obtained in which the conformation of the enzyme appears “open”, allowing entry of DNA, as required for the initiation of transcription. By contrast, a previous crystal form contained the enzyme in a “closed” conformation, appropriate for retention of DNA during RNA chain elongation. Interaction with two polymerase subunits, Rpb4 and Rpb7, favors the closed conformation, and binding of general transcription factor TFIIE may do so as well. The effect of Rpb4 and Rpb7, together with previous biochemical evidence, leads to the conclusion that the open to closed transition is a crucial step in the transcription initiation process.

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Introduction

The initiation and elongation phases of transcription place very different requirements on the transcription apparatus. DNA must be able to enter the template binding site during initiation but should not leave during elongation. A conformational change of DNA and RNA polymerases has been suggested to accommodate these dual requirements. Structural studies have disclosed multiple conformations of the polymerases and have revealed a mobile region responsible for the interchange between them.

The first of a dozen structures so far determined was that of a 75 kDa fragment of *Escherichia coli* DNA polymerase I from X-ray diffraction at 3.3 Å resolution (Ollis *et al.*, 1985). A notable feature was a cleft about 25 Å in diameter, appropriate for binding duplex DNA. A break in the chain where about 50 residues could not be traced was attributed to a mobile loop, suggested to allow entry of DNA in the cleft and then to reach around and retain it during elongation. The generality of this mechanism was indicated by the structure of *E. coli* RNA polymerase, a multisubunit enzyme of 450 kDa (Darst *et al.*, 1989), determined by electron crystallography at about 25 Å resolution. A cleft of similar size and shape was observed, with an arm of protein density in the location of the missing loop from the DNA polymerase I structure, possibly visible in the RNA polymerase structure due to the lower resolution of the electron crystallographic analysis. Subsequent studies of a series of single subunit DNA and RNA polymerases by

X-ray diffraction, and of multisubunit RNA polymerases, including the giant eukaryotic enzymes, by electron crystallography, have confirmed the generality of the cleft and added important details (Davies *et al.*, 1994; Kim *et al.*, 1995; Kohlstaedt *et al.*, 1992; Korolev *et al.*, 1995; Polyakov *et al.*, 1995; Sawaya *et al.*, 1994; Schultz *et al.*, 1993; Sousa *et al.*, 1993, 1994; Darst *et al.*, 1991a). The X-ray structures have shown a conserved fold in the vicinity of the cleft, with three catalytic aspartate residues common to all the enzymes located in the floor of the cleft. These findings, together with structures of cocrystals containing template DNA and substrate nucleoside triphosphate (Beese *et al.*, 1993; Pelletier *et al.*, 1994), have definitively established the location of the active center of the DNA-dependent polymerases in the 25 Å cleft.

The role of a flexible arm of protein density in clamping DNA in the cleft and enhancing the processivity of chain elongation is supported by several lines of evidence (Beese *et al.*, 1993; Polyakov *et al.*, 1995; Sawaya *et al.*, 1994; Sousa *et al.*, 1994). Structures of *E. coli* DNA polymerase I and rat DNA polymerase beta in replicating complexes show the arm surrounding DNA in the cleft. In the case of *E. coli* RNA polymerase, structures of holo- and core enzymes, which function in initiation and chain elongation, respectively, exhibit different conformations of the arm: an “open” conformation in the holoenzyme exposes the cleft to solution, enabling entry of DNA during initiation; a “closed” conformation in the core polymerase, with the cleft enveloped by protein, leaves no gap

for release of DNA, assuring its retention during elongation (Polyakov *et al.*, 1995).

The structure of eukaryotic (yeast) RNA polymerase II determined previously (Darst *et al.*, 1991a) resembles that of the *E. coli* core enzyme, with a closed conformation of the arm around the active center cleft. On this basis, it was suggested that the polymerase II structure represents the elongating form of the enzyme (Polyakov *et al.*, 1995). Evidence for polymerase II in an open conformation, appropriate for initiation has, however, been lacking. We report here on a new crystal form of polymerase II from which such evidence has been obtained.

Results

Novel crystal form of $\Delta 4/7$ RNA polymerase II in ammonium acetate

The structure of RNA polymerase II was determined previously from two-dimensional (2-D) crystals of a mutant form of the yeast enzyme lacking two small subunits, Rpb4 and Rpb7. Study of the mutant enzyme was necessitated by a substoichiometric amount of Rpb4 and Rpb7 in wild-type enzyme isolated from logarithmically grown cells, resulting in heterogeneity that interfered with crystallization. The mutant ($\Delta 4/7$) enzyme is unable to initiate transcription except upon addition of the missing subunits, but is fully active in chain elongation. Crystallization was performed in the presence of 60 mM ammonium sulfate, which is inhibitory to initiation, but which allows chain elongation. The 2-D crystals obtained were in the two-sided plane group $c2$, with two polymerase molecules in the center of the unit cell, related by a dyad axis between them in the plane of the crystal. As mentioned above, the polymerase structure derived by electron crystallography appeared to represent the elongating form of the enzyme.

We investigated polymerase crystallization in the presence of ammonium acetate, which is more conducive to transcription initiation than ammonium sulfate, and obtained a different crystal form, in the two-sided plane group $p1$. The frequency of crystallization in this $p1$ (acetate) form was much less than that of the $c2$ (sulfate) form, precluding study of unstained specimens, but the crystals were sufficient in size (1 to 2 mm in extent) and number (found on about 10% of grids examined) for analysis in negative stain. The presence of ammonium acetate interfered somewhat with negative staining by uranyl acetate, but no other compound could be found that gave better results. Diffraction in stain extended to about 25 Å, and a projected structure was obtained (Figure 1) showing antiparallel rows of polymerase molecules that, in contrast with the $c2$ (sulfate) crystal form, were all identically oriented with respect to the lipid layer.

Comparison of the projected structures from $p1$ (acetate) and $c2$ (sulfate) forms in stain (Figure 2)

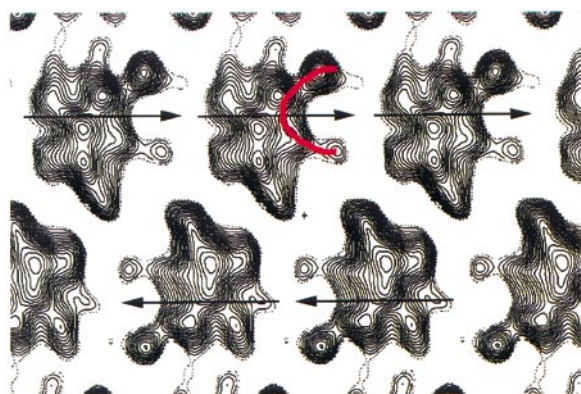


Figure 1. Contour plot of an electron density projection map of a $p1$ (acetate) crystal (unit cell parameters $a = 133.2$ Å, $b = 269.9$ Å, $\beta = 111.1^\circ$) preserved in negative stain. The $\Delta 4/7$ polymerase monomers are arranged in antiparallel rows, all interacting with the charged lipid layer through the same face of the molecule. The monomers are in the "open" conformation, and the DNA-binding channel is fully accessible. The arrows are included to indicate the orientation of the monomers in the 2-D crystal, and the arrowheads correspond to the approximate location of the DNA-binding cleft. The red "arch" superimposed on one of the polymerase molecules traces the position of domains that define the open DNA cleft.

revealed little difference except in the vicinity of the presumed active center cleft (arrowheads in Figure 1). The arm of density surrounding the cleft in the $c2$ (sulfate) form is largely absent from the $p1$ (acetate) form. The $p1$ (acetate) structure appears to represent an open conformation of the polymerase, with the cleft accessible for entry of DNA. This analysis assumes that penetration of the polymerase molecule by stain is not substantially altered by the change in buffer conditions,

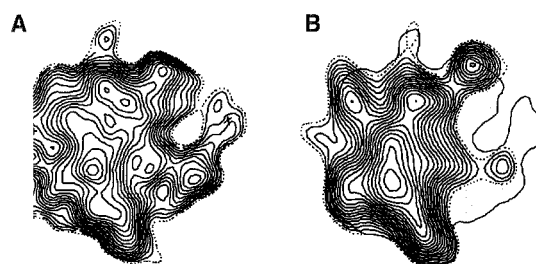


Figure 2. Comparison between the electron density projection maps for the closed (A, $c2$ (sulfate) crystals), and open (B, $p1$ (acetate) crystals), superimposed on the outline (shaded in gray) of the projection map for the closed conformation) enzyme conformations of $\Delta 4/7$ RNA polymerase II. Both projection maps were calculated from crystals preserved in uranyl acetate. Note that after visual alignment the maps coincide very closely, except for the right-hand side of the molecule. The differences are most dramatic in the area where the DNA-binding cleft is located, and in the area along the proposed DNA path on the polymerase (Leuther *et al.*, 1996).

and that the polymerase molecules are identically oriented in the p1 (acetate) and c2 (sulfate) crystal forms. Both assumptions appear justified by the similarity of the two projected structures: after translational and rotational alignment, the two structures could be nearly perfectly superimposed, except in the region of the cleft. The absence in the p1 (acetate) form of the arm of density that blocks access to the active center cleft in the closed conformation is not merely due to differences in staining, as packing of the molecules along a row is very tight, and a rearrangement of the crystal would be required to accommodate that domain (see results for the wild-type enzyme below). Further support for similarity in orientation came from rotating a 3-D structure of the $\Delta 4/7$ enzyme to find a view nearly identical with the projected structure of the wild-type enzyme in p1 (acetate) form (see below). The rotation matrix for obtaining the best match entailed an in-plane rotation of about 40° (counterclockwise), and a rotation out of the plane of the crystal by less than 10° . This out-of-plane rotation produces minimal changes in the region of the cleft, and cannot account for the differences between the open (p1 acetate) and closed (c2 sulfate) structures.

Influence of transcription factor IIE upon RNA polymerase II crystallization

Previous studies of general transcription factor TFIIE - $\Delta 4/7$ polymerase II cocrystals (c2 (sulfate) form) revealed interaction of TFIIE with the polymerase arm in the closed conformation (Leuther *et al.*, 1996). We attempted formation of TFIIE - polymerase II cocrystals under acetate conditions and obtained none of the p1 (acetate) form but only crystals in the c2 (sulfate) form. This effect of TFIIE may be due either to stabilization of the closed conformation or to steric hindrance of p1 (acetate) lattice formation. Evidence in favor of stabilization of the closed conformation comes from the effect of TFIIE upon the frequency of crystallization. As mentioned above, crystallization of the $\Delta 4/7$ polymerase in acetate is comparatively rare. Addition of TFIIE caused a marked increase in the number of crystals obtained, and these were invariably in the c2 (sulfate) form. This influence of TFIIE is not due to additional crystal contacts in the c2 (sulfate) form (Leuther *et al.*, 1996), and so can be attributed only to an effect upon polymerase conformation.

Crystallization of wild-type RNA polymerase II in ammonium acetate

RNA polymerase II isolated from stationary phase yeast cells possesses a full complement of Rpb4 and Rpb7 (Choder & Young, 1993). This fully stoichiometric wild-type enzyme forms 2-D crystals under sulfate conditions comparable in quality to those obtained from the $\Delta 4/7$ polymerase



Figure 3. Contour plot of an electron density projection map of the p1 (acetate) crystals (preserved in amorphous ice) formed by wild-type RNA polymerase II. The unit cell parameters for these (acetate) crystals are $a = 125.4 \text{ \AA}$, $b = 227.5 \text{ \AA}$, $\beta = 106.1^\circ$. As was observed for crystals of the $\Delta 4/7$ enzyme formed under the same buffer conditions, the crystal is composed of antiparallel rows of monomers, all interacting with the charged lipid layer through the same face of the molecule. However, comparison with the projection map shown in Figure 1 indicates that the wild-type enzyme is in the closed conformation, and that there has been a packing rearrangement to accommodate the extra density present in the plane of the crystal. As indicated by the arrows, all the monomers have rotated $\sim 30^\circ$ clockwise with respect to the orientation observed when the enzyme is in the open conformation, and the protein density immediately behind the arrowheads (under the green portion of the arch that traces the position of the domains that define the DNA-binding cleft) blocks access to the DNA-binding channel.

(unpublished results). The wild-type enzyme also crystallized under acetate conditions, more reproducibly than the $\Delta 4/7$ polymerase, allowing examination of unstained specimens. The wild-type polymerase crystals were in the two-sided plane group p1, but were not identical with the p1 (acetate) crystals of the $\Delta 4/7$ molecule described above. The projected structure of the wild-type enzyme (Figure 3) revealed a closed conformation of the arm around the active center cleft, and a rotation in the plane of the crystal by about 30° relative to the $\Delta 4/7$ molecule in the p1 (acetate) form. The closed conformation of the arm appeared to necessitate the in-plane rotation, as additional space is required to accommodate localization of the arm density in the plane of the crystal without interference with the adjacent molecule in the p1 (acetate) packing arrangement.

Identification of the wild-type structure in acetate with the closed conformation previously seen for the $\Delta 4/7$ structure in sulfate was confirmed by computation of a real-space difference map (Figure 4). The two structures were visually aligned, and the absence of extensive difference density around the periphery of the molecule, as well as the localized nature of the differences observed, attest to the quality of the alignment and

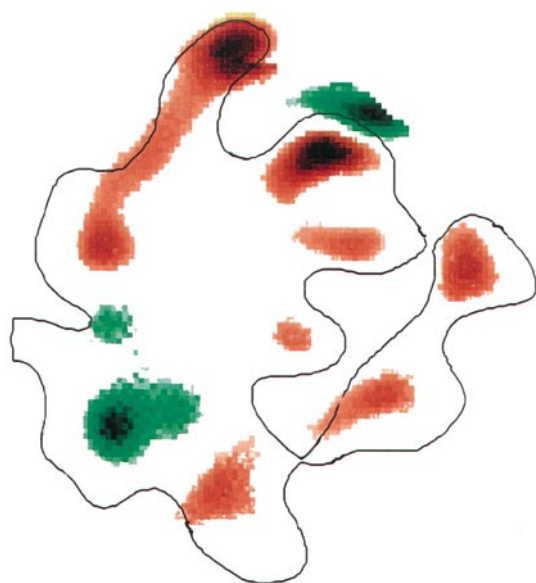


Figure 4. Electron density changes (within two standard deviations of the mean value) observed when the electron density projection map calculated from $\Delta 4/7$ RNA polymerase II crystals (c2 (sulfate) form, enzyme in the closed conformation) is subtracted from the electron density projection map for the wild-type enzyme (p1 (acetate) form, also in the closed conformation). Both maps were calculated from specimens preserved in amorphous ice. Positive differences are shown in green, negative differences are shown in red.

to the lack of significant out-of-plane rotation between the two structures. The comparatively small differences observed may be attributed to differences in subunit composition (presence of subunits 4 and 7 in the wild-type enzyme), to small differences in conformation, and to differences in mobility between corresponding domains in the wild-type and $\Delta 4/7$ enzymes.

Discussion

Our chief finding is the occurrence of a second (open) conformation of $\Delta 4/7$ yeast RNA polymerase II, differing from that previously observed (closed) by the apparent absence of density due to the arm surrounding the active center cleft. It is clear that the shift between the open and closed conformations is not simply a result of changes in crystal packing, since the closed conformation can be accommodated in a p1 lattice (first observed for the open conformation) by an in-plane rotation of the molecule, seen in the case of wild-type polymerase II. Association of $\Delta 4/7$ RNA polymerase II with TFIIE or with the polymerase subunits Rpb4 and Rpb7 favors the closed conformation. The effect of TFIIE can be understood in terms of its interaction with the arm around the cleft in the closed conformation, demonstrated previously (Leuther *et al.*, 1996).

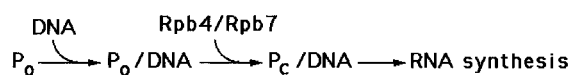


Figure 5. Pathway of transcription initiation showing only the steps relevant to this work. RNA polymerase binds DNA in the open conformation of the enzyme (P_o). Rpb4 and Rpb7 (which may enter following DNA binding or be present throughout) may act during the transition from open to closed (P_c) conformations or in a subsequent step. Regardless, the open to closed transition is a prerequisite for the start of RNA synthesis.

The effect of Rpb4 and Rpb7 on polymerase conformation was seemingly inconsistent with biochemical studies of $\Delta 4/7$ RNA polymerase II. We previously found that the $\Delta 4/7$ enzyme was unable to initiate transcription in a promoter-dependent system *in vitro*, and that addition of Rpb4 and Rpb7 restored activity (Edwards *et al.*, 1991). On the other hand, the $\Delta 4/7$ polymerase was indistinguishable from wild-type enzyme in RNA chain elongation. Rpb4 and Rpb7 are evidently involved in transcription initiation which, as mentioned above, requires the open conformation of the polymerase for entry of DNA to the active center cleft. How might this requirement be reconciled with the demonstration here that Rpb4 and Rpb7 favor the closed polymerase conformation? We suggest that Rpb4 and Rpb7 participate in a step of the initiation process following polymerase-DNA interaction (Figure 5). This step may be the open to closed polymerase transition, or it may be a subsequent step in the initiation process. The simplest possibility is that Rpb4 and Rpb7 are absent from the enzyme at the time of DNA binding and become associated at a later stage. Indeed, exchange of these subunits between polymerase molecules has been inferred from previous evidence (Edwards *et al.*, 1991). Regardless, we are led to the conclusion that the open to closed transition occurs prior to the start of RNA synthesis. A similar conclusion has been reached for the initiation of *E. coli* RNA polymerase transcription (Polyakov *et al.*, 1995).

Materials and Methods

RNA polymerase preparation and crystallization

Wild-type and $\Delta 4/7$ RNA polymerase II were isolated and purified as described (Edwards *et al.*, 1991). Preparation of two-dimensional crystals on charged lipid layers was also carried out as described (Asturias & Kornberg, 1995; Darst *et al.*, 1991b). The crystallization buffer contained 100 mM ammonium acetate, 50 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM spermidine, 5 mM dithiothreitol. Yeast RNA polymerase II aliquots (80 to 100 $\mu g\ ml^{-1}$) were incubated in Nylon wells for 9 to 12 hours, and then "loop transferred" (Asturias & Kornberg, 1995) to electron microscope grids. After transfer, the crystals were preserved using uranyl acetate

as a negative stain, or by freezing in amorphous ice (Dubochet *et al.*, 1988).

Data collection and processing

Depending on the preservation method, the samples were mounted in either a standard or a cold microscope stage (model 626-DH, Gatan, Inc., Pleasanton, CA). Data were collected using Philips CM 12 (LaB₆ filament source), and CM 20 (field emission gun source) microscopes. Electron micrographs were recorded using Kodak SO-163 film (Eastman Kodak Co., Rochester, NY), digitized using a PDS flatbed microdensitometer (Perkin Elmer, South Pasadena, CA), and then subjected to electron crystallographic analysis using the standard set of programs developed at the Medical Research Council (Crowther *et al.*, 1996), after appropriate modifications to accommodate the large unit cell size of our crystals. Data collection and processing will be described elsewhere in greater detail.

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