Simple Elastic Network Modeling Studies Provide Insights into Possible Motions of Multi-subunit RNA Polymerases

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1 Introduction

Although several crystallographic structures of multi-subunit RNA polymerases have been solved, its exact mechanism is yet to be understood. The existing structures as well as the complex processes catalyzed by these enzymes imply that they undergo considerable conformational changes while transcribing genetic information from DNA to RNA. Here we describe new motions of multi-subunit RNA polymerases that have been observed through simple elastic network modeling studies. We examined *Saccharomyces cerevisiae* and *Thermus thermophilus* RNA polymerase crystal structures by using elastic normal mode analysis as implemented in the program NORMA[1]. Besides the previously described motions, we observed new ones that seem common to structures of both bacterial and yeast RNA polymerases. One of these motions is a rotation of two major domains of the polymerase in opposite directions around the bridge helix, an α-helix that spans the center of the enzyme. This motion results in an overall twisting of the whole structure which causes partial unfolding of the bridge helix center. In addition, the clamp opening and closing is also accompanied by bending of the bridge helix. It is reasonable to believe these motions play an important role in the RNA polymerase mechanism during transcription elongation for a few reasons. First, these are motions that are seen in multiple structures at low frequency modes. Second, the bridge α-helix has highly conserved residues in the center with low helix propensities (helix breakers). Finally, Some crystal structures show a slightly bent bridge helix near the region in contact with the template DNA strand. These findings provide support for previous suggestions that bending of the bridge helix may facilitate translocation of the DNA, which is a critical step in RNA synthesis.

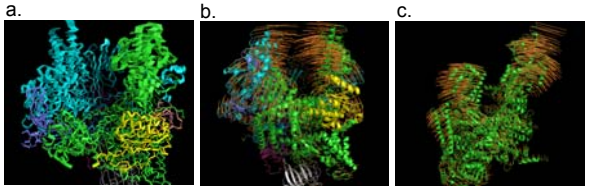
2 What is an RNA Polymerase?

RNA Polymerase is a multi-subunit enzyme that synthesizes RNA in all living cells through a process known as transcription.

- **Bacteria**: One RNA polymerase.
  - Synthesizes all three types of RNA for the cell.
  - Made of six subunits $\alpha_2\beta\beta'\omega$ and σ.
  - σ subunit (factor) dissociates from the polymerase after transcription is initiated.
- **Eukaryotes**: multiple RNA polymerases.
  - RNA polymerase I (rRNA), RNA polymerase III (tRNA/rRNA).
  - **RNA polymerase II**: synthesizes mRNAs.
    - Twelve Subunits :Rpb1 through Rpb12.
    - Many transcription factors such as TFIID, TFIIE, TFIIF, TFIIH modulates transcription.
    - Transcription elongation mechanism is controversial.

3 Polymerase Motions Also Observed in the Past

a. b. c.



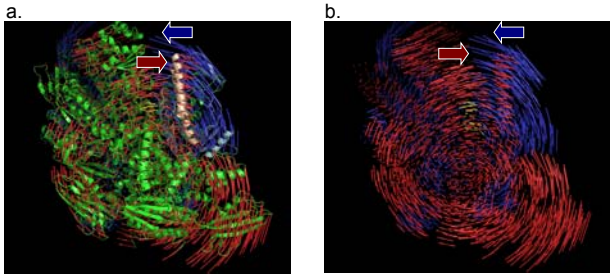
- Opening and closing motions of the RNA polymerase clamp has been found in NMA of *Saccharomyces cerevisiae* (a and b.) as well as *Thermus thermophilus* (c.) crystal structures[2,3].

4 Elastic Network Modeling and Analysis

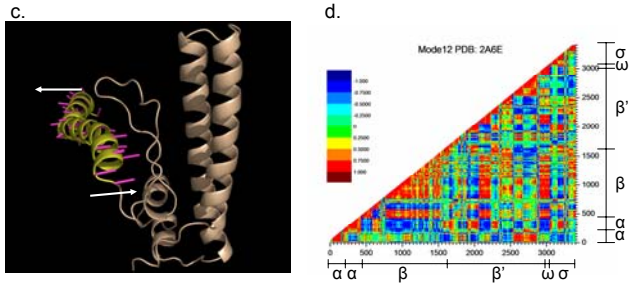
1. Utilized NORMA, an elastic network modeling software developed by Karsten Shure and colleages[1] to predict possible large scale motions of multi-subunit RNA polymerases.
2. Analyzed the results from NORMA by studying the motions of different modes of different structures.
   - Cross-correlation plots to determine which domains move together.
   - Vector diagrams to illustrate the direction and relative amplitudes of the motions with the original structure set as a starting point.

5 Bacterial RNA Polymerase Motions

a. b.



c. d.



a. Circular motions of the *Thermus Thermophilus* RNA polymerase. Two separate domains of the enzyme are moving in opposite directions, creating an overall twisting motion around the area of the bridge α-helix.

b. Circular motions of the RNA polymerase from the side emphasizing on the vector directions.

c. Slight bending motion of the bridge α-helix is observed when the two domains are moving in opposite directions (figure a. and b.).

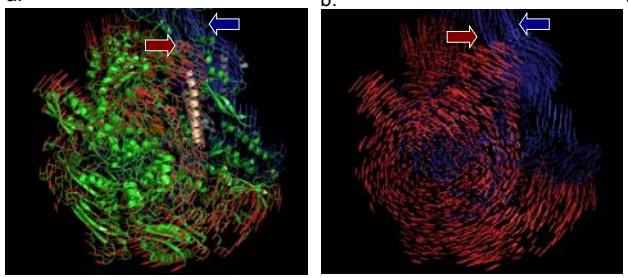
d. Cross-correlation plot of mode12. This plot also helped us analyze the motions of the RNA polymerases. The red regions represent completely correlated motions (blocks moving in the same direction) and the blue regions represents anti-correlated motions (blocks moving in opposite directions.

All motions here are from mode 12 of NORMA analysis freq: 58.02 cm^{-1}

PDB: 2A6E

6 Yeast RNA Polymerase II Motions

a. b.



c.

a. and b. are from mode 8 of PDB 2E2H freq: 73.9 cm^{-1}

c. is from mode 11 of PDB 2E2H freq: 91.9 cm^{-1}

a. Circular motion of two separate domains of the yeast RNA polymerase II. There are two domains in this particular mode that move in opposite directions resulting in a twisting motion, similar to the bacterial polymerase. This motion also contributes to slight bending of the bridge α-helix.

b. Circular motion of the RNA polymerase II identical to figure a. but emphasizing the vector directions.

c. Slight bending motions of the bridge α-helix. The center of the helix is steady while the two sides of the helix move in opposite directions. This motion supports the idea that bending of this α-helix may contribute to forward movement of the DNA.

7 Conclusions

1. Low frequency modes of normal mode analysis showed common motions among different crystal structures.
   - Opening and closing motions of the enzyme was detected in our studies similar to other studies[2,3].
   - New motions such as the rotation of opposite sides in different directions were observed.
   - Slight bending of the bridge α-helix was also observed.
2. Common motions seen among multiple structures suggest that it may be an important motion of the polymerase.
3. To get more details on the exact role of the motions of the bridge-helix and others, normal mode trajectories will be used in the future to guide molecular dynamic simulations.
   - Our hope is that these modeling studies will provide new insights into the mechanism of the multi-subunit RNA polymerase transcription and will help us design new experiments to further understand it's function

1. Suhre et al., *Acta Cryst. D*, 62:1098, 2006.
2. Wynsberghe et al., *Biochemisry*, 43:13083, 2004.
3. Delarue et al., *JMB*, 320:1011, 2002.