Single Molecule Experiments of RNA polymerase in DNA Transcription

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RNA polymerase plays a central role in transcription, which makes a RNA copy of a DNA sequence, and thus leads the genetic codes towards protein, the main component of living creatures. When the transcription begins, the RNA polymerase opens up a local region of the double helix of DNA and exposes the nucleotides on a short stretch on each strand. One of the exposed DNA strands servers as a template for complementary base pairing with incoming ribonucleoside triphosphate monomers (NTPs). The RNA polymerase molecule then moves along the DNA, unwinding the DNA helix just ahead to expose a new region of the template strand for complementary base pairing. In this way, the growing RNA chain is extended by one nucleotide at a time in the 5'-to-3' direction [1]. Although these are all known textbook literatures, detailed features in RNA polymerase mechanism are remained unknown. One of the key issues is the nature of the single reaction step that lead to movement of the RNA polymerase. The understanding of this mechanism may leads to the control of DNA transcription, which may be essential to the remedy of genetic diseases.

In order to examine the transcription process closely, Guthold et al.[2] used scanning force microscope (SFM) to observe the transcription of *Escherichia coli* RNA Polymerase (RNAP) directly. They fixed the RNAP-DNA complex on the mica surface, and use SFM to take sequential images of the DNA, which is sliding on the surface while being transcribed. (Fig.1). Although the high spatial resolution of SFM enables them to look at the transcription process directly, the low time resolution of SFM (about 50 seconds per image of 256*256 pixels) greatly limited the accuracy of their measurement. In order to slow down the rate of transcription, they lower the concentration of NTPs to 5 μM. According to the Michaelis-Menten model, the steady state reaction rate is given by

$$v = v_{\max} \frac{[NTP]}{[NTP] + K_m}.$$

Where Km is the Michaelis constant. With a maximum velocity of v_{max}=50 nt/s [3] and K_m=50μM [2], a rate of v=5 nt/s is obtained. But their assay yielded an average transcription rate of only 1.5+/-0.8 nt/s. The reason of the difference is that both the RNAP/DNA complex and DNA chain are confined in the mica surface. Therefore, the reaction cannot be fitted into Michaelis-Menten model. As a result, their SFM data consists with their biochemical transcription assay on mica surface, which gives a rate of around 1 nt/s.

Although this experiment visualized the relative translocation between RNAP and DNA in transcription, the fact that both the RNAP/DNA complex and the DNA chain are absorbed onto a mica surface may not reveal the *in-vivo* process faithfully. A more delicate experiment was carried out by M. D. Wang et al. in 1998 [4]. In their assay, the ternary complex consisting of RNAP, the DNA template, and a nascent RNA chain is fixed to a coverglass surface inside a flow cell. A bead is attached to the transcriptional

down stream end of the DNA, and held up by an optical trap (Fig.2). When the DNA is being transcribed, the polymerase will pull the bead away from the trap center. Two different working modes of the optical trap are used in their experiment. One is called "open loop" mode, which maintains constant stiffness (force on the bead over the displacement from the trap center), and another is called "closed loop" mode, which can clamp the position of the bead through a feedback circuit, when the bead is drawn to the edge of the optical trap. When the bead is moved out of the range of the trap (open loop mode), or the bead is stalled (close loop mode), the trap is repositioned manually to continue the measurement. Benefiting by the optical trap and the interferometer, the position of the bead and the pulling force can be measured simultaneously in high time resolution. Therefore, they derived a single molecule force-velocity (F-V) curve (Fig. 3). The curve shows that the velocity remains nearly independent of the load until it approaches to the stall force, which is quite different from the F-V curve of another molecular motor, kinesin, which has a nearly linear F-V curve [5].

This optical trap experiment measured the force and transcription rate in the same time. And the DNA molecule is held up by the optical trap so its movment is not confined by the surface. Therefore, it is a more faithful representation of the *in vivo* transcription. But, since the DNA molecule has a topology of high link number, either the RNA polymerase or the DNA itself must rotate in order to release the stress during the process of transcription. Yet neither of these two experiments can reveal the rotational motion.

In order to measure the rotational motion in transcription, Harada at el. use magnetic beads and fluorescent beads to observe the rotation directly [6]. In their experiment, a DNA template of 4,971 base pairs (1.7 μm) long was constructed, the RNA polyermase is attached to the glass surface, and a magnetic bead of diameter 850 nm coated with fluorescent beads (streptavidin) is attached to the downstream end of the DNA. The magnet holds the magnetic bead vertically at a force of ~0.1pN, which confines the rotation in a horizontal plane and does not prevent the bead from rotation (Fig 4). Then, after adding NTPs, the rotation of the DNA can be directly visualized by a high-speed camera. Thus the rate of rotation can be obtained. Since the template DNA is right-handed double helix, thus has about 10.4 base pairs per helical turn[1]. Therefore the translocation rate (nt/s) divided by 10.4 will be the rotation rate (revolutions/second), if the polymerase tracks along the DNA helix faithfully. The comparison of the ration rate and the translocation rate in solution, and on individual molecules of RNAP on the glass surface[7] shows that this seemed to be the case at [NTP] below ~20μM (Fig. 5).

The inconsistence of the rotation rate and translocation rate at high NTP concentration shows some uncertainty in whether the RNA polymerase tracks the DNA chain faithfully. Thus assays that measure rotation and translocation simultaneously may provide further information on this question. One possible experiment scheme may be the marriage of Harada et. al. and Wang et al.'s experiment. We can align the cover glass vertically, and use optical trap to pull the DNA horizontally (Fig.6). At the same time, attach daughter fluorescent dyes to the main bead. By choosing the wavelength of the laser, the optical trap can also excite the dyes. Since most fluorescent dyes have separations on excitation and emission wavelengths, the signal of the fluorescence dye can be distinguished and

detected by a camera. Therefore, the force, translocation rate, and rotation rate can be determined simultaneously.

In summary, recent single molecule RNA polymerase experiments reveals many important aspects of the DNA transcription process, such as translocation rate, rotation rate, force-velocity curve, rotation torque and so on. Yet whether RNA polymerase rotates around DNA or vice versa, or even exist other mechanisms [8] remains unknown. And the individual rotation-translocation step of the transcription is not resolved neither. Therefore, further single molecule experiments with different schemes and smaller beads may be helpful to answer these questions.

Figures:

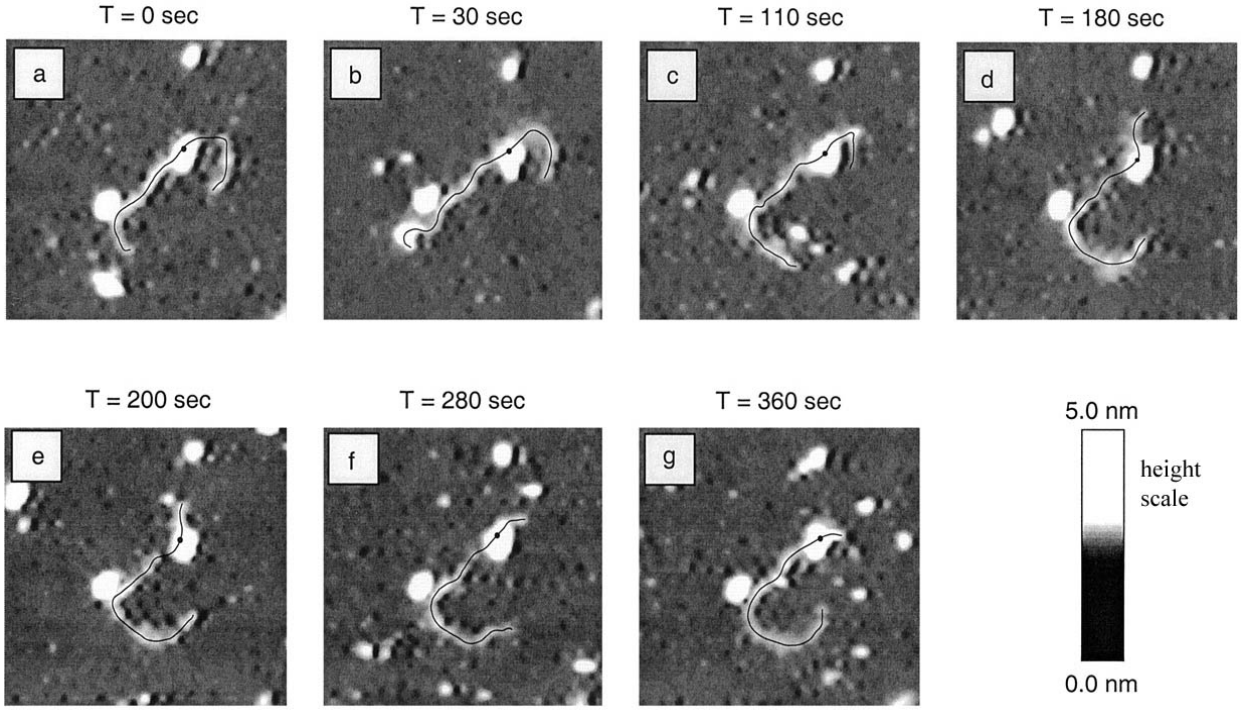

Fig.1. The image sequence of a single transcribing RNA polymerase molecule. [NTP]=5μM.

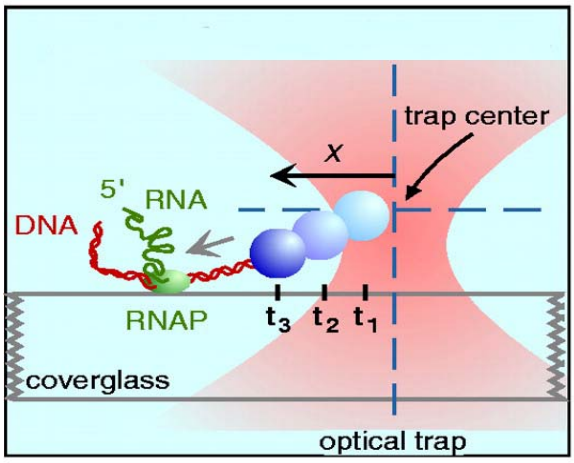

Fig.2. Using the optical trap to measure the translocation of the bead when RNAP transcribing the DNA. The complex consists of the RNAP, the DNA template, and a nascent RNA chain if fixed to a coverglass surface inside a flow cell. A polystyrene bead attached to the downstream end of the DNA is captured and held under low tension by the optical trap. The displacement of the bead, x, is detected by interferometrically. As the transcription proceeds, DNA is drawn through the polymerase, pulling the bead from the trap center at successive times t_1, t_2, and t_3.

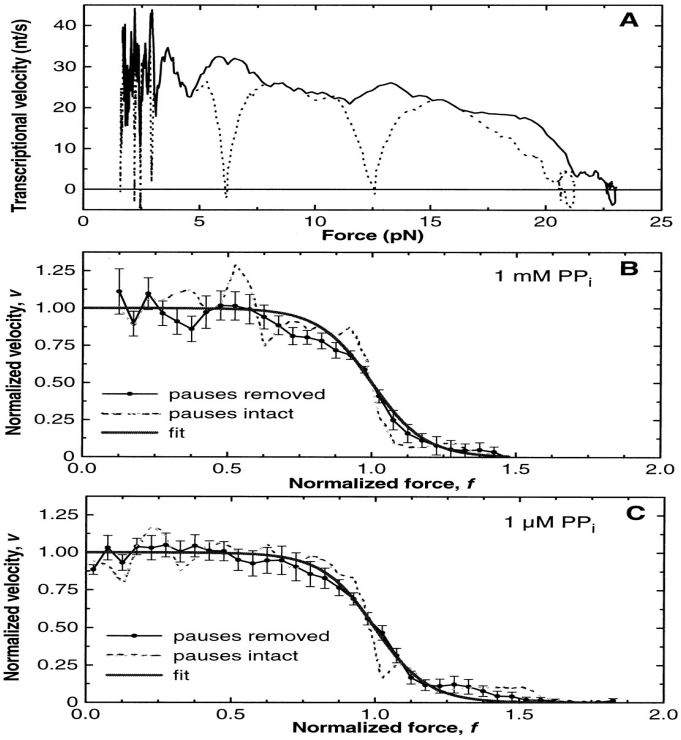

Fig. 3. The force-velocity relationship for RNAP. (A) example of a single-molecule F-V curve. (B) Ensemble average for eight complexes in 1 mM NTP, 1 mM PPi. (C) Ensemble average for 13 complexes in 1 mM NTP, 1 μM PPi. In both (B) and (C), the velocities are normalized to the unloaded speed V_0, and the forces are normalized to the force at half maximum velocity $F_{1/2}$.

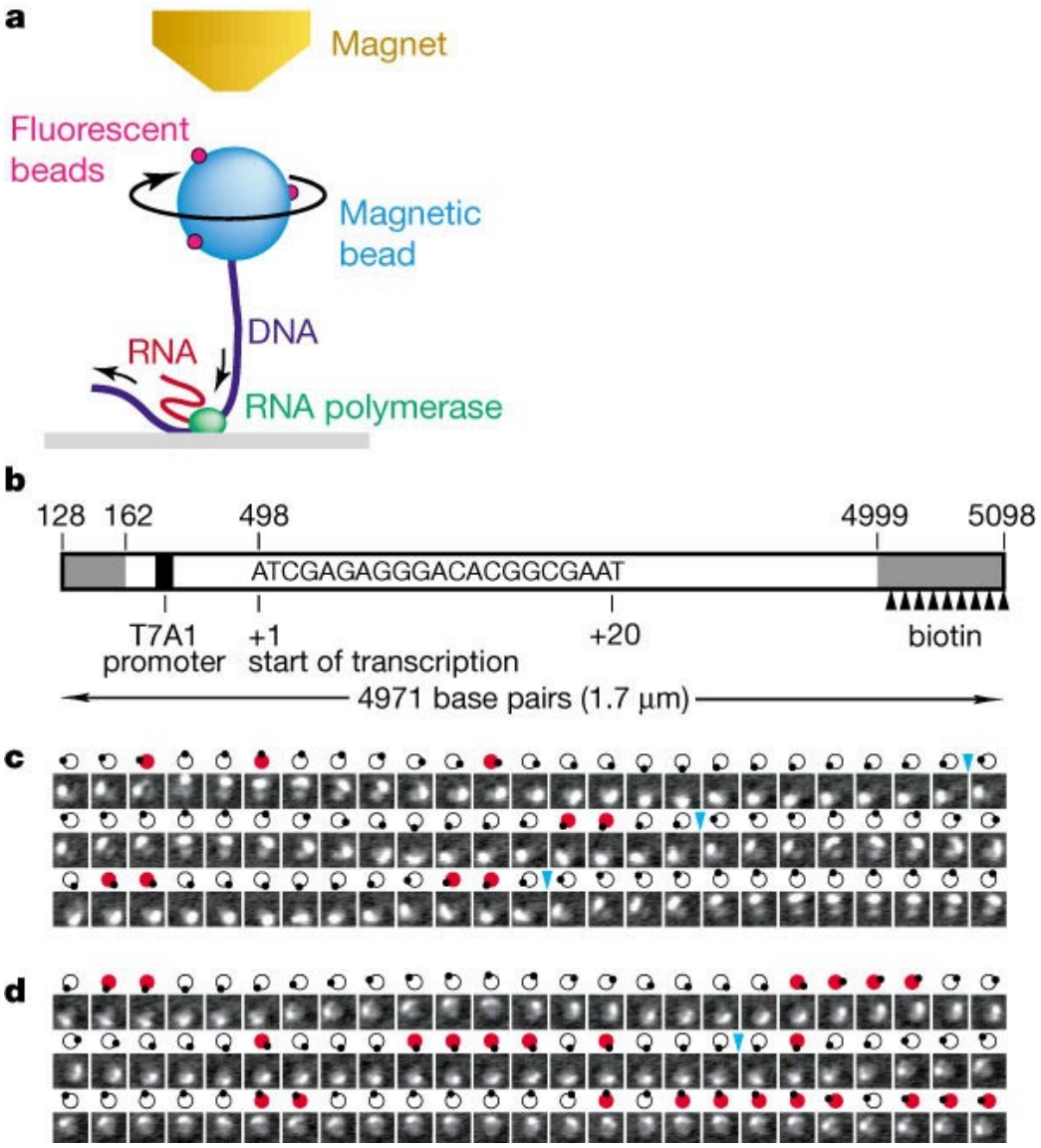

Fig. 4. Observation of DNA rotation by RNA polymerase. **a**, Observation system (not to scale). The magnetic bead was pulled upwards by a disk-shaped neodymium magnet. Magnetization was vertical and did not prevent bead rotation. Daughter fluorescent beads served as markers of rotation. **b**, The DNA template. Numbers above are from the T7 D111 sequence. Rotation assay started from position +20. The magnetic bead was attached to the nine biotins. Shaded ends denote primers for the polymerase chain reaction. **c**, **d**, Snapshots of rotating beads at 133-ms intervals at NTP concentrations of 50 µM (**c**) and 2.5 µM (**d**). Grey part at the center, a magnetic bead visualized with transmitted light; moving white spot, a daughter fluorescent bead or probably its aggregates. Diagrams show their relative positions. Blue arrowheads indicate completion of a turn. Red diagrams show moments of anticlockwise rotation due to Brownian fluctuations of DNA; these were less noticeable at high [NTP], presumably because supercoiling had reduced the effective tether length. Image size, 2.4 × 2.4 μm^2.

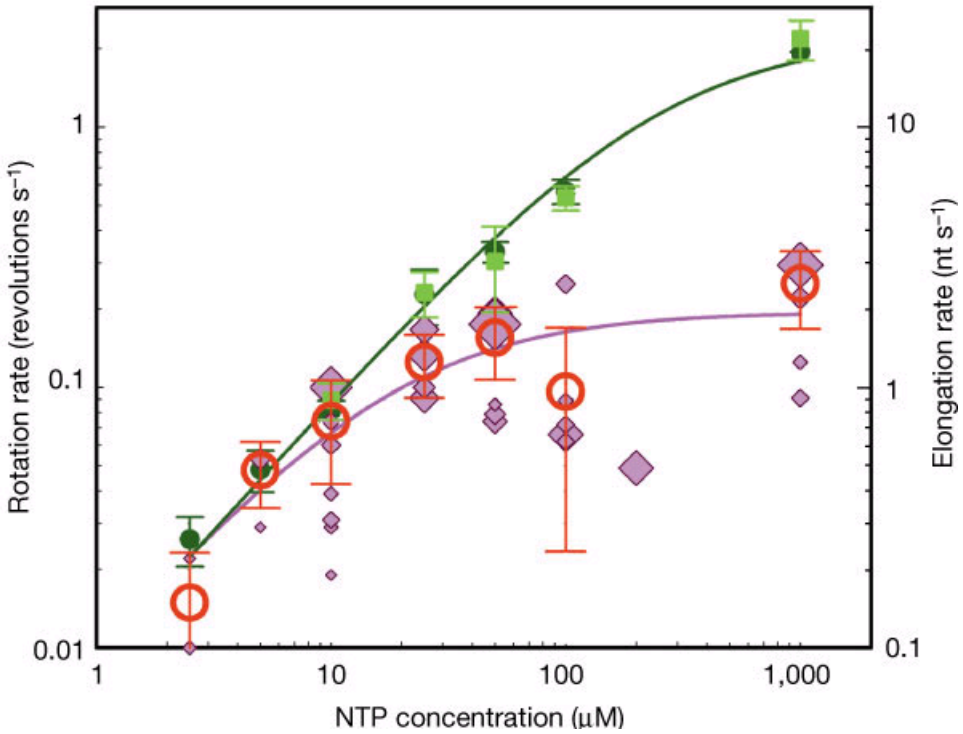

Fig. 5. Comparison of rotation and elongation rates. Purple diamonds, rotation rates for individual beads, estimated as the total number of revolutions N divided by time; symbol size ⌢ $N^{1/2}$. Red circles, rotation rates at indicated [NTP] averaged with weights ⌢ N. Dark green circles, elongation rates in solution; light green squares, elongation rates for individual RNA polymerase molecules on the glass surface.

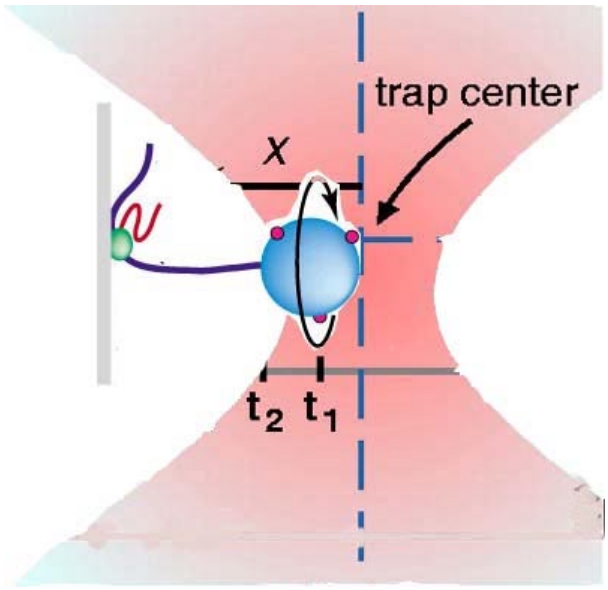

Fig.6. My proposal of the experimental scheme. The cover glass is aligned vertically, and the optical trap pulls the bead and stretch the DNA chain horizontally. Fluorescence dyes are attached on the bead, so that the rotation of the bead can be detected by a camera

on the right hand side. At the same time, the force can be measured by the optical trap and the displacement can be measured by the interferometer.

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