

## Facilitated Target Location on DNA by Individual *Escherichia coli* RNA Polymerase Molecules Observed with the Scanning Force Microscope Operating in Liquid\*

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In *Escherichia coli*, transcription is carried out by a single enzyme, *E. coli* RNA polymerase (RNAP),<sup>1</sup> and the rate of transcription initiation is controlled, in part, by the rate at which the polymerase can find the promoter. One problem that RNAP, and for that matter most other specific DNA-binding proteins, must solve is how to overcome the kinetic barrier of finding its specific binding site amid a large excess of nonspecific DNA. In 1968, Adam and Delbrück (1) proposed that the efficiency of a diffusion-controlled search could be enhanced by orders of magnitude if it were to take place in a space of lower dimensionality. In 1970, Riggs *et al.* (2) reported that *E. coli lac* repressor locates its target site at rates up to 1000 times faster than what could be accounted for by a three-dimensional diffusion-controlled search. These authors suggested that, because the enzyme has some affinity for non-promoter DNA, their observations could be explained if the enzyme could bind nonspecifically anywhere to the DNA and then move in a one-dimensional random walk along the DNA until it finds its target. This finding sparked the interest of scientists to demonstrate the existence and to elucidate the mechanisms of facilitated target location in DNA-binding proteins.

Two investigations have used kinetic analysis to obtain evidence that RNAP can locate a promoter by one-dimensional diffusion along nonspecific DNA. Singer and Wu (3) employed a rapid mixing/photocross-linking method to monitor the time-dependent density of bound RNAP along a relaxed, circular DNA plasmid containing a single promoter. It was found that the occupancy by RNAP of the DNA segments near a promoter decreased faster than the occupancy of the segments farther away from the promoter. This phenomenon was interpreted as evidence that RNAP reached the promoter through one-dimensional diffusion along the DNA. Fitting the data to a theoretical model that included RNAP sliding, a dissociation rate constant of  $k_{\text{off}} = 0.3 \text{ s}^{-1}$  and a one-dimensional diffusion coefficient of

$D_{1D} = 1.5 \times 10^{-9} \text{ cm}^2/\text{s}$  for RNAP sliding along DNA were determined. The resulting average lifetime of a nonspecific complex,  $\tau_{\text{av}} = 3.3 \text{ s}$ , is, however, about 1000 times larger than the one found in electron microscopy experiments (4). In the second study, Ricchetti *et al.* (5) measured the occupancy of DNA fragments carrying A1 promoters as a function of the length of the downstream and upstream flanking sequences. It was found that a longer downstream flanking sequence increased the occupancy of the adjacent promoter in agreement with the sliding model. However, upstream sequences had surprisingly little effect on promoter occupancy.

In two recent studies, fluorescence microscopy was used to observe the interactions of fluorescently labeled RNAP with DNA. Kabata *et al.* (6) utilized superintensified fluorescence microscopy to visualize the movement of RNAP over  $\lambda$ -DNA combs. A fraction of the RNAP molecules was seen to deviate from the direction of bulk flow and to move along the extended DNA molecules. This observation suggests that RNAP can slide along nonspecific DNA. However, in this experiment the RNAP was propelled predominantly by flow and was, therefore, not driven by thermal motion. In the second study, Harada *et al.* (7) used internal reflection fluorescence microscopy to observe the dissociation and association events of RNAP with different regions of a single  $\lambda$ -DNA molecule, which was suspended in laser tweezers. For AT-rich regions fast and slow dissociation constants of 3.0 and 0.66  $\text{s}^{-1}$ , respectively, were determined; and for GC-rich regions a fast dissociation rate of 8.4  $\text{s}^{-1}$  was measured. In a few instances sliding of RNAP along the DNA was also observed. However, the occurrence of these events was rare because the present spatial resolution of this technique is about 200 nm, and the typical sliding range of a one-dimensionally diffusing RNAP is presumably less than this limit. Nevertheless, a one-dimensional diffusion constant of  $\sim 10^{-10} \text{ cm}^2/\text{s}$  was estimated from these data.

Other mechanisms of facilitated target location besides *sliding* have been proposed, as depicted in Fig. 1. One mechanism involves the transfer of DNA-binding proteins from one segment of DNA to another. This process, known as *intersegment transfer* (8), could make it more likely for a protein molecule to bind to distant regions in the genome by decreasing the effective volume of diffusion and thus increasing the target location rate. Moreover, as pointed out by von Hippel and Berg (8), the efficiency of this process can be enhanced if the energy barrier of intersegment transfer is lower than the barrier for the dissociation-association cycle. In particular, the high local concentration of DNA would also make contact increasingly likely and multiple transfer events possible.

A third proposed mechanism is the *intradomain association and dissociation* or “*hopping*,” of the protein along the DNA (8). In this process, the protein-DNA interactions may consist of the protein bouncing along the DNA until it finds the target site or completely dissociates from the DNA. The target search would then be accelerated in a manner similar to sliding, with a reduced number of sampled sequences but presumably higher kinetic barriers.

### Scanning Force Microscopy Studies

This review will center on the capability of the scanning force microscope (SFM) to operate in liquids to directly visualize the movements of *E. coli* RNA polymerase on nonspecific DNA, thus providing direct evidence for the mechanisms of facilitated

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<sup>1</sup> The abbreviations used are: RNAP, RNA polymerase; SFM, scanning force microscope; bp, base pairs.

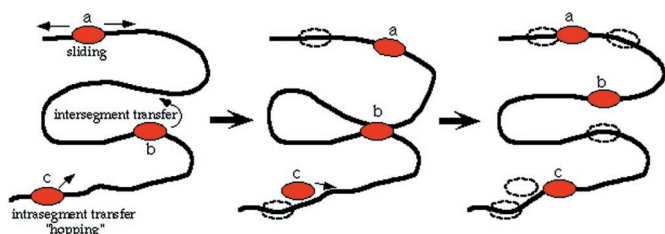


FIG. 1. Schematic of the proposed mechanisms of facilitated target location, where the ovals represent RNA polymerase molecules and the curved line represents the DNA. *a*, sliding is the one-dimensional diffusion of the RNAP along the contour of the DNA. *b*, intersegment transfer is the translocation of the RNAP from one point of the DNA to another distant point through the formation of a ternary structure. *c*, intradomain association and dissociation or "hopping" is the movements of the RNAP relative to the DNA through a series of collisions on a small scale.

target location. By necessity, this review will focus on experiments performed mainly in our laboratory, but the results will be compared with those obtained by other techniques. In experiments of this kind, the DNA molecules are deposited on a mica surface, where they interact with other molecules (*i.e.* RNAP) present in the solution covering the surface. To study these interactions properly it is necessary to understand the state of the DNA on such surfaces and to control the mobility of the DNA molecules.

**DNA Diffusion on Mica Surface**—Imaging the dynamic interactions between RNAP and DNA by SFM requires first finding conditions in which the protein-DNA complexes are adsorbed stably enough to be imaged by the scanning tip. Yet, these same conditions must allow the molecules to bind loosely enough to be able to diffuse on the substrate. Deposition conditions had to be found to reconcile these conflicting requirements. As reported previously (9–11), binding of DNA to the mica surface requires the presence of a divalent cation such as  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Ca^{2+}$ ,  $Zn^{2+}$ , or  $Ni^{2+}$  in the deposition buffer. Magnesium was chosen as the divalent ion in the experiments to be described herein, as it is also essential for RNAP activity, whereas other cations are inhibitory to transcription ( $Zn^{2+}$ ,  $Ni^{2+}$ ) (12) or induce kinks in the DNA ( $Zn^{2+}$ ) (13). Images were acquired under liquid using tapping mode SFM. Electron beam-deposited tips (14) were used in all experiments. These deposition and imaging conditions favor the binding of the DNA molecules to the mica, while allowing them to diffuse laterally.

In Fig. 2, the contours of free DNA molecules (1047 bp) from 11 sequentially recorded images are superimposed to illustrate the DNA motion on the mica surface. The excursion of the center of mass of one of the DNA molecules is shown in the *inset* of Fig. 2. The average two-dimensional diffusion coefficient, determined from 91 molecules observed in several different experiments, had a value of about  $7 \times 10^{-14} \text{ cm}^2/\text{s}$ . The diffusion coefficient varied by approximately 1 order of magnitude among different experiments. This is probably because of the local variability of the mica surface, which influences the strength of the mica-DNA interactions and thus the mobility of DNA.

**Diffusion of RNA Polymerase along Nonspecific DNA**—Having obtained conditions in which DNA molecules can diffuse on the substrate surface while remaining adsorbed to it, experiments were designed to investigate whether RNAP can diffuse one-dimensionally along nonspecific DNA. The remainder of this article will be devoted to describing those experiments and analyzing the results in comparison with the limited amount of information available in the literature. Complexes of RNAP holoenzyme formed on a promoterless DNA fragment were deposited onto mica and imaged in buffer with the SFM. Fig. 3

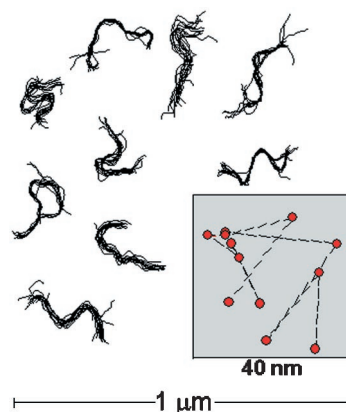


FIG. 2. Two-dimensional diffusion of DNA on mica. The contours of 9 DNA molecules (1047 bp) obtained from 11 consecutive SFM images (time interval between images,  $\sim 40 \text{ s}$ ) are superimposed to illustrate the diffusion of the molecules on the surface. The *inset* shows the movement of the center of mass of one representative DNA molecule. To eliminate the *x-y* drift of the microscope between frames, the movement of the center of mass of each molecule was normalized to a fixed reference point on the surface.

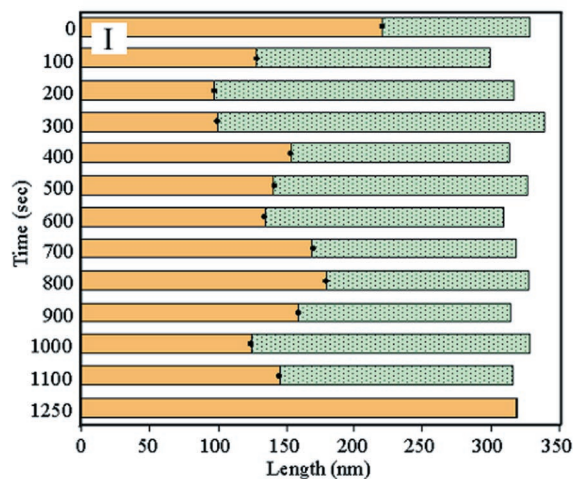
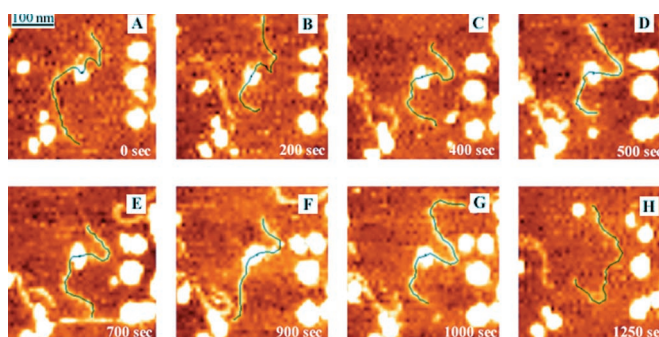


FIG. 3. One-dimensional diffusion of nonspecific binary complexes of RNAP along the DNA. A–H, sequentially acquired SFM images showing an RNAP molecule sliding on DNA. Polymerase appears to slide back and forth several times along the DNA molecule before it is released. The elapsed time after the initial observation of the complex is indicated in each image. The DNA contour is traced with a *thin line*. I, bar plot representation of the length of the two DNA arms (dotted and solid bars) in 13 sequential images.

shows a time-lapse sequence of a nonspecific RNAP-DNA complex. At the center of each image the polymerase appears as a white globular feature stably bound to the surface. The DNA molecule appears to be sliding back and forth beneath the enzyme (Fig. 3, A–G). In the last frame (Fig. 3H), the protein has released the DNA. The position of the RNAP on the DNA



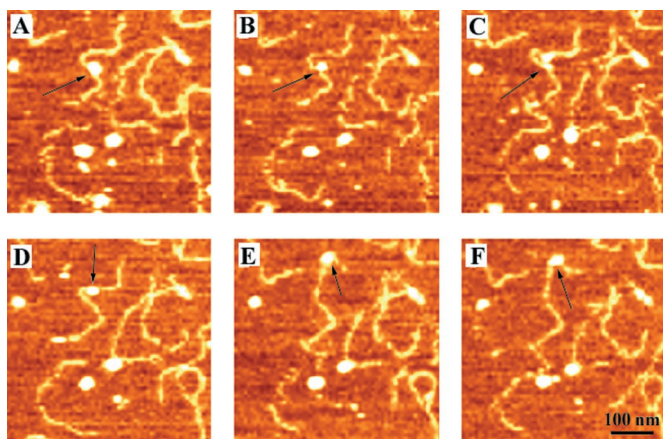


FIG. 4. **Intersegment transfer.** An RNA polymerase core enzyme (indicated by the *arrow*) is transferred from one segment of the DNA to another about 100 bp apart through the formation of a ternary structure (C). After the transfer, the protein continued to slide. The time lapse between consecutive images is around 40 s.

fragment in successive images is depicted schematically in Fig. 3I. Similar plots, obtained from over 30 different imaging sequences, revealed that the relative position of the protein on the DNA is consistent with the pattern of a random walk in one dimension. The average one-dimensional diffusion coefficient obtained from these images has a value of  $D_{1D} = 1.1 \times 10^{-13}$  cm<sup>2</sup>/s. The average lifetime of these nonspecific RNAP-DNA complexes on the surface was about 600 s, which is large compared with nonspecific complexes in solution (see below).

**Effect of Heparin on Nonspecific Complexes**—To demonstrate that the observed RNAP-DNA complexes are indeed nonspecific complexes, and not just the DNA and RNAP positioned in close proximity by chance, a control experiment was carried out by introducing heparin into the liquid chamber. Heparin, a polyanion and DNA analog, is known to disrupt the relatively weak nonspecific RNAP-DNA interaction and to inhibit DNA binding of RNAP (15–17). Accordingly, if these complexes were true nonspecific complexes, heparin should have a deleterious effect on sliding. On the other hand, heparin would have *no* effect if RNAP and DNA were not interacting with each other or if the complexes were the heparin-resistant tight binding kind (15). When 14 sliding, nonspecific complexes were exposed to heparin, 13 of them dissociated right after the arrival of heparin in the liquid chamber, and no re-association was observed; only one of them seemed to stay bound (data not shown). These results showed that heparin significantly reduced the average lifetime of the observed DNA-RNAP complexes, providing additional proof that they were indeed nonspecific complexes.

**Intersegment Transfer**—Fig. 4 shows a series of images where RNAP core enzyme combines sliding with an *intersegment transfer* event. Intersegment transfer is not nearly as common as sliding, probably because of the low concentration of DNA on the surface. The images show an intermediate structure consisting of an RNA polymerase molecule binding two segments of the same DNA molecule (Fig. 4C). An interesting feature of the *intersegment transfer* event is the formation of a tight hairpin turn in conjunction with the formation of the ternary complex.

**Intradomain Association and Dissociation (Hopping)**—The images in Fig. 5 show that an RNA polymerase molecule, initially bound to the DNA, dissociated from the DNA and then re-associated. The series of events depicted by these images indicate that, in the process of searching for the promoter, the polymerase might associate to and dissociate from the DNA repeatedly until the target site is found. At present, the tem-

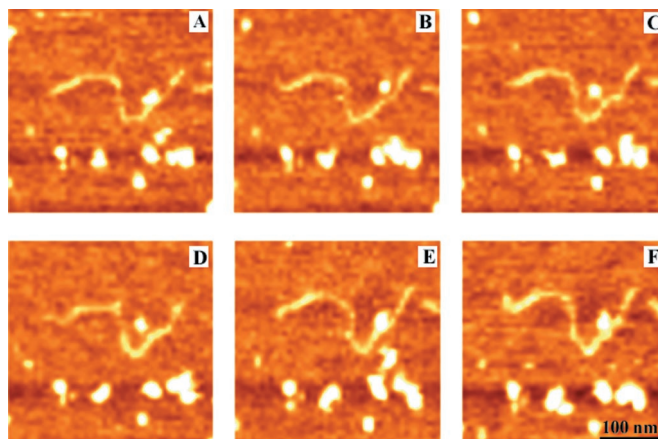


FIG. 5. **Intradomain association and dissociation or “hopping.”** The bound RNA polymerase first dissociated from the DNA (B) and then associated again (E). The time lapse between consecutive images is around 40 s.

poral resolution of the SFM does not allow for a more detailed observation of these events. The rates of association and dissociation of the RNA polymerase in solution are much higher than the time resolution of the images, and it is possible that between images multiple association-dissociation cycles occur.

### Conclusions and Summary

In the present study, sequential SFM images of single RNAP molecules moving (*sliding*, *intersegment transfer*, and possibly *hopping*) along individual DNA molecules were recorded and analyzed. These images demonstrate that RNAP can use one-dimensional diffusion along nonspecific DNA, as well as *intersegment transfer* and *hopping*, to accelerate the search for promoters. The ability of heparin to disrupt the sliding process and, thereby, reduce the mean diffusion time of the polymerase on the DNA supports the identification of the RNAP-DNA complexes as nonspecific complexes and eliminates the possibility that these complexes are simply DNA and RNAP juxtaposed by chance. Furthermore, this observation indicates that the sliding, nonspecific complexes are not the tight binding type previously described for polymerase (15) because those complexes cannot be dissociated by heparin.

The average lifetime of surface-bound nonspecific complexes was found to be about 600 s, which is much larger than the average lifetime of 3.3 s (3) and 1.5–0.1 s (7) reported for nonspecific complexes in solution under similar salt conditions. A possible explanation is that the constraints imposed by the surface slow down the rates at which the complex adopts intermediate configurations required for dissociation. Dissociation rates smaller by a factor of 180 would require an increase in the activation energy leading to this intermediate by about 3.1 kcal/mol. This value is well within the range of activation energies for surface diffusion of DNA (~8.1 kcal/mol) obtained from a comparison between the diffusion constants of the DNA fragment on the surface,  $D_{2D} = 7 \cdot 10^{-14}$  cm<sup>2</sup>/s, and in solution,  $D_{3D} = 5.4 \cdot 10^{-8}$  cm<sup>2</sup>/s (18). Furthermore, both molecules remain in close proximity to each other for a longer time when RNAP releases the DNA, because both of them are adsorbed to the surface. Attractive interactions between the molecules might increase the probability that RNAP and DNA re-associate many times before the DNA fragment eventually dissociates completely and diffuses away. This interpretation is consistent with the observation that the lifetime of nonspecific complexes is reduced in the presence of the negatively charged DNA competitor, heparin. It is possible that the small diffusion constants and large lifetimes observed on the surface might also be

observed in analogous experiments that were carried out in very viscous medium such as the inside of cells (19).

In summary, images obtained with tapping-mode SFM could demonstrate the diffusion of *E. coli* RNA polymerase along DNA. Moreover, direct evidence of other mechanisms of facilitated targeting of RNAP such as *intersegment transfer* and possibly *hopping* (*intradomain association and dissociation*) was obtained for the first time. Ternary intermediates, in which RNAP appears to be simultaneously bound to two DNA segments, were directly observed during *intersegment transfer* events. In addition, these transfer events were preceded and followed by sliding processes in some experiments. A combination of *intersegment transfer*, *hopping*, and *sliding* should result in a more effective search of the promoter. Even though it is not possible to directly compare numerical data from the SFM study with kinetic data from solution studies, the current analysis provides the first direct evidence on individual complexes, at high resolution, for these targeting mechanisms. Future developments in SFM technology should allow faster scanning of biological samples and thereby improve the temporal resolution of these SFM movies. These improved capabilities, together with single-molecule analysis in real time, will make it easier to use SFM to visualize complex biological processes as they occur, one molecule at a time.

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