Tumor Suppressor p53 Slides on DNA with Low Friction and High Stability

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ABSTRACT The p53 protein, a transcription factor of key importance in tumorigenesis, is suggested to diffuse one-dimensionally along DNA via its C-terminal domain, a process that is proposed to regulate gene activation both positively and negatively. There has been no direct observation of p53 moving along DNA, however, and little is known about the mechanism and rate of its translocation. Here, we use single-molecule techniques to visualize, in real time, the one-dimensional diffusion of p53 along DNA. The one-dimensional diffusion coefficient is measured to be close to the theoretical limit, indicative of movement along a free energy landscape with low activation barriers. We further investigate the mechanism of translocation and determine that p53 is capable of sliding—moving along DNA while in continuous contact with the duplex, rather than through a series of hops between nearby bases.

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The tumor suppressor p53 is a transcription factor that responds to stresses, such as DNA damage, oxidative stress, heat shock, and deregulated oncogene expression, by inducing cell-cycle arrest or apoptosis (1). The protein binds nonspecific DNA through its highly basic C-terminus domain (2) and can undergo one-dimensional diffusion on DNA using this domain (3). This one-dimensional diffusion has been suggested to regulate gene activation both positively and negatively. Experiments that have examined the dissociation of p53 from short DNA have shown that deleting the C-terminus (1,3–5) or replacing it with the neutral C-terminus of the related p73 protein (4) slows the dissociation of p53 from its promoter. Moreover, for wild-type p53, blocking the ends of the DNA (3), circularizing the DNA (3), or increasing the length of the DNA (5,6) slows the rate of dissociation, suggesting that p53 relies on one-dimensional diffusion along DNA to escape from its promoter. On the other hand, forms of p53 that are missing the C-terminus activate target genes in vivo much more slowly and lack the capacity to resist tumor transformation of cell lines (6). These results are consistent with recent theoretical work that point to both a negative regulatory effect of excessive nonspecific binding through sequestration of transcription factors from their cognate sites and a positive effect of one-dimensional diffusion as part of a mechanism that can greatly reduce the time needed for a transcription factor to reach its promoter (7–10).

The molecular mechanism underlying one-dimensional translocation of p53 along DNA is poorly understood. Two distinct scenarios have been proposed: a sliding mode that involves a constant protein-DNA contact, and a hopping mechanism that consists of repeated rounds of dissociation and reassociation at a nearby location (11). A high probability of rebinding close to a site of dissociation (12) makes discrimination between the two mechanisms challenging. To distinguish between these two translocation mechanisms, a direct observation of the movement of p53 along DNA is needed. Recent advances in fluorescence imaging have allowed the visualization of individual proteins diffusing along stretched DNA molecules (13,14). Here, we report the observation of one-dimensional diffusion of individual p53 proteins along stretched DNA and demonstrate that the protein slides along DNA while maintaining a physical contact with the duplex. We present a quantitative analysis of its diffusion properties and arrive at a description of the free energy landscape underlying the protein's motion.

We fluorescently labeled full-length, human p53 and used total internal reflection fluorescence microscopy to visualize its movement along individual λ-phage DNA molecules (Fig. 1, *A–D*; and see Supplementary Material, Data S1). The DNA was tethered with one end to a surface and mechanically stretched by applying a laminar flow of aqueous buffer exerting a hydrodynamic drag force on the DNA duplex (14). The fluorescence of the proteins was imaged on a charge-coupled device and their positions tracked by determining the Gaussian-fitted center of the single-molecule intensity profiles (15). Fig. 1 *D* shows a time series of fluorescence images indicating the movement of an individual p53 along the DNA. Two example trajectories of the movement of individual proteins along the DNA are shown in Fig. 1 *E*. The mean-square displacement (MSD) versus

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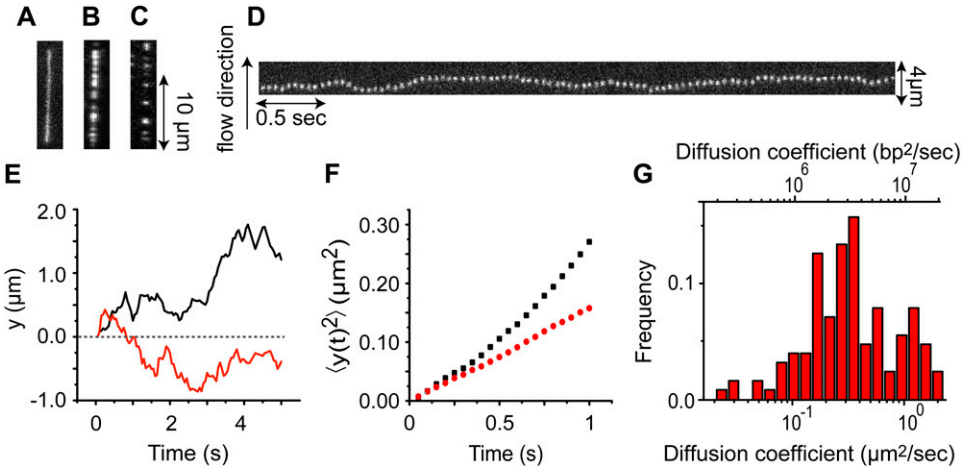

FIGURE 1 (*A*) Stained λ DNA molecule stretched by flow. (*B* and *C*) Images of p53 proteins on DNA. Protein concentration is 0.3 nM; the total salt concentration is 75 mM in panel *B* and 125 mM in panel *C*. (*D*) Kymograph of an individual fluorescently-labeled p53 protein moving on flow-stretched DNA (protein concentration is 5 pM). (*E*) Diffusion trajectories of two p53 proteins. (*F*) Mean-square displacement (MSD) versus time of the same two trajectories. (*G*) Histogram of diffusion coefficient *D* of 162 individual p53 proteins (125 mM total salt concentration; similar distributions were observed with other salt concentrations; see Data S1).

time for the same trajectories is shown in Fig. 1 *F*. To estimate the diffusion coefficient of the p53 motion along DNA, we first correct for a drift component in the trajectories due to the hydrodynamic drag exerted by the flow of the buffer on the protein. We do this by subtracting the mean drift over all trajectories (weighted by their durations) from each individual trajectory (see Data S1) (16). The diffusion coefficient for each trajectory then can be calculated by determining the slope of the MSD versus time (see Data S1). We observe a diffusion coefficient of $(2.60 \pm 2.17) \times 10^6$ bp^2/s, and a drift velocity of 262 ± 1144 bp/s. Fig. 1 *G* shows a histogram of diffusion coefficients of 162 individual p53 molecules. The large standard deviations do not reflect experimental errors, but describe the width of the measured distributions of diffusion coefficients and drift velocities for many molecules.

Next, we determined whether p53 is moving while maintaining continuous contact with DNA (i.e., sliding) or whether it translocates by making small but frequent hops off and back onto the DNA (i.e., hopping). Since protein affinity to nonspecific DNA is determined primarily by electrostatic interactions, varying the salt concentration in the experiments can modulate these interactions and allow us to discriminate between the hopping and sliding models (14,17). If a hopping process causes one-dimensional diffusion, a higher salt concentration will lower the nonspecific binding affinity, increasing the fraction of the time the protein spends in solution, and effectively increasing the measured diffusion coefficient. Conversely, a sliding process will result in a diffusion constant that is independent of the salt concentration. Fig. 2 *A* (*open blue triangles*) shows that the one-dimensional diffusion constant is entirely insensitive to the salt concentration, which rules out the hopping mechanism and leaves sliding as the only plausible mechanism of p53's one-dimensional translocation. In both the sliding and hopping scenarios, the thermodynamic binding affinity of the protein to the DNA is expected to decrease with increasing salt concentration. As a proxy for affinity, we measure the total

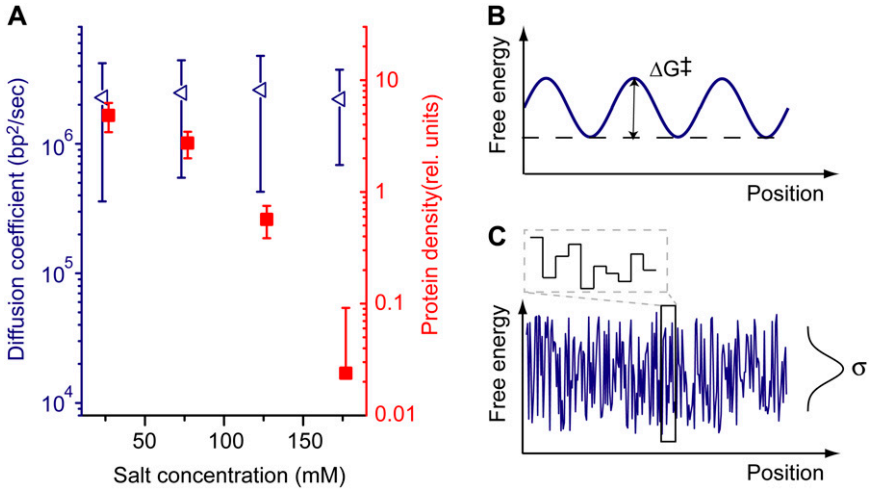

FIGURE 2 (*A*) Diffusion coefficient *D* (*blue triangles*) and protein density on DNA (*red squares*) as a function of salt concentration. Protein density is measured as the number of observed proteins per kbp of DNA. (*B*) Iso-energetic model to describe translocation of protein along DNA. For each base-pair, the protein has to overcome an energy barrier of height $\Delta G^{\ddagger}$. (*C*) Random energy model. Sequence-dependent energies of protein-DNA complex over the length of the DNA follow a Gaussian distribution with variance σ^2.

number of proteins bound to the DNA at various salt concentrations (Fig. 1, *B* and *C*; Fig. 2 *A*, *solid red squares*) and observe the expected decrease at higher salt concentrations.

By comparing the experimentally measured diffusion coefficient with the theoretical maximum value for the limiting case of zero protein-DNA friction, we can obtain quantitative information about the free energy landscape of sliding. For a globular protein the size of p53, we estimate the upper limit of the diffusion coefficient to be $D_{\mathrm{lim}} = 7.7 \times 10^6$ bp^2/s (Data S1) (17). Our measured diffusion coefficient of $D_{1\mathrm{D}} = (2.60 \pm 2.17) \times 10^6$ bp^2/s is a factor of 3.6 below this limit. We consider two models that describe this protein-DNA friction.

In the first model, protein-DNA binding energy is constant across all positions (on nonspecific DNA), but translocating a distance of one basepair requires overcoming a free energy barrier of a constant height $\Delta G^{\ddagger}$ (Fig. 2 *B*).

In the second model (Fig. 2 *C*), the energy of protein-DNA binding varies with the sequence and is normally distributed with variance σ^2, making sliding along DNA a random walk in a random energy landscape. Using the first model, the relation $\langle x^2 \rangle = 2D_{\mathrm{limit}}t$, and the assumed step size of 1 bp, we obtain a theoretical upper limit for the stepping rate $k_{\mathrm{lim}} = 1.54 \times 10^7$ s^{-1}. From the measured diffusion constant we obtain the stepping rate $k_{\mathrm{exp}} = (5.20 \pm 4.34) \times 10^6$ s^{-1}. The Arrhenius relation $k_{\mathrm{exp}}/k_{\mathrm{lim}} = \exp(-\Delta G^{\ddagger}/k_{\mathrm{B}}T)$ provides a value of 1.78 ± 1.21 $k_{\mathrm{B}}T$ for the activation barrier $\Delta G^{\ddagger}$.

Previous theoretical work demonstrated that the second model yields diffusive behavior with the diffusion coefficient $D_{1\mathrm{D}} = D_{\mathrm{ideal}}(1 + \sigma^2\beta^2/2)^{1/2}\exp(-7\sigma^2\beta^2/4)$, where $\beta = 1/k_{\mathrm{B}}T$ (15). Using this equation we obtain $\sigma = 0.84 \pm 0.40$ $k_{\mathrm{B}}T$. Values obtained from the two models are similar and provide a picture of diffusion on a fairly smooth energy landscape, consistent with previous theoretical results that rapid search is possible only with energy barriers <2 $k_{\mathrm{B}}T$ (11).

We offer the first direct experimental observation of sliding on DNA by p53, and indeed by any eukaryotic transcription factor. One-dimensional sliding is physically necessary for the mechanism of facilitated diffusion, which allows for rapid binding in vivo of transcription factors to their promoters. Further studies will address whether one-dimensional sliding of p53 reported here contributes to facilitated promoter search, a mechanism that is suggested to be available to prokaryotes (17). Our work opens the way for better understanding of the role of nonspecific protein-DNA binding and sliding in negative and positive regulation of gene expression and, broadly, the physical bases of gene regulation. Future work will examine the role of the various p53 domains and modifications in modulating the kinetics of protein-DNA interactions.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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