**Sequence-dependent sliding kinetics of p53**

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Proper timing of gene expression requires that transcription factors (TFs) efficiently locate and bind their target sites within a genome. Theoretical studies have long proposed that one-dimensional sliding along DNA while simultaneously reading its sequence can accelerate TF's location of target sites. Sliding by prokaryotic and eukaryotic TFs were subsequently observed. More recent theoretical investigations have argued that simultaneous reading and sliding is not possible for TFs without their possessing at least two DNA-binding modes. The tumor suppressor p53 has been shown to slide on DNA, and recent experiments have offered structural and single molecule support for a two-mode model for the protein. If the model is applicable to p53, then the requirement that TFs be able to read while sliding implies that noncognate sites will affect p53's mobility on DNA, which will thus be generally sequence-dependent. Here, we confirm this prediction with single-molecule microscopy measurements of p53's local diffusivity on noncognate DNA. We show how a two-mode model accurately predicts the variation in local diffusivity, while a single-mode model does not.

Further evidence that the recognition by p53 of its targets and the timing thereof can depend on its noncognate binding properties and its ability to change between multiple modes of binding, in addition to the much better-studied effects of cognate-site binding.

**T**umor suppressor p53 is known as the “guardian of the genome.” The protein is mutated in more than 50% of cancers (1), and plays important roles in activating DNA-repair, cell-cycle arrest, and apoptosis. To prevent the replication of damaged DNA, damage-activated p53 must reach its target promoters sufficiently fast.

In addition to its clinical importance, p53 is the first eukaryotic transcription factor (TF) directly observed to undergo one-dimensional (1D) diffusion on DNA (2). This 1D sliding has long been proposed to facilitate search by DNA-binding proteins (DBPs) (3, 4) and was characterized by single-molecule experiments for RNA polymerase (5, 6), mitochondrial repair enzymes (7), lac repressor (8, 9), and repair complex Msh2–Msh6 (10) (see ref. 11 for review). Sliding of p53 was first demonstrated by bulk biochemical experiments and was shown to play a role in p53's activation of target genes (12, 13). Several theoretical and experimental studies (7, 8, 14, 15) have shown that despite a vast excess of accessible DNA ($10^{17}$–$10^{19}$ bp) to which DBPs have nonspecific affinity, their search process can be efficient if they alternate rounds of 1D sliding while bound nonspecifically with rounds of three-dimensional (3D) diffusion between different sections of DNA. Until recently, in vivo studies have been limited to bacterial systems (8), and it remained unclear whether the same mechanism was at play in eukaryotes where DNA is packed by nucleosomes limiting space for sliding. Recently Larson et al. (16) have demonstrated that yeast DBPs search for their sites by a 1D/3D mechanism. By demonstrating the ability of p53 not only to slide but also to “read” DNA while sliding, our study provides strong support for a 1D/3D mechanism in high eukaryotes. An earlier single-molecule study of Harada et al. (6) found sequence-dependence in the dissociation kinetics of RNA polymerase, but not in its sliding kinetics.

For sliding to be functional in facilitating search, DBPs must be able to read the DNA sequence they slide on. This implies that the binding energy at each DNA position depends on the sequence. The magnitude of this sequence dependence can be captured by the standard deviation of the energies comprising the landscape, $\sigma$. Prior theoretical work (17) demonstrated that $\sigma \leq 1.5 k_B T$ is required for fast sliding and facilitated search, while stability of the protein–DNA complex requires $\sigma \geq 5 k_B T$. These mutually unsatisfactory requirements lead to an apparent “speed-stability paradox” (17, 18), which had been qualitatively anticipated (19).

A multimode model protein–DNA interaction has been proposed to resolve this paradox (17, 18): In their simplest form, DBPs exhibit two modes of binding and stochastically switch between them: a search ($S$) mode, and a recognition ($R$) mode (Fig. 1 $A$ and $B$). In $S$ mode, DBPs bind with small sequence-dependence ($\leq 1.5 k_B T$) and slide efficiently. In $R$ mode, DBPs bind highly specifically and sliding is negligible. Fast search requires the $S$ mode to have significantly lower average energy and thus be favored at nearly all DNA positions to avoid unproductive visits to $R$ mode (18). Transition into $R$ mode at cognate-like sites that have low energy slows down sliding, but allows recognition of the cognate site. The central idea of this study is that such slowdown on near-cognate sites can be detected experimentally.

Experimental evidence supporting this multimode model includes NMR studies that found specific and nonspecific conformations for lac repressor (20, 21) on DNA, and X-ray crystallography of the restriction enzyme BstYI (22). While an NMR study of homeodomain hopping and sliding on DNA found only small structural changes between specific and nonspecific binding, the homeodomain is only weakly specific, with only 200-fold preference for its cognate site over nonspecific DNA (23), compared with p53's ~10$^5$-fold preference (24). Transcriptional activation by the yeast TF Mbp1 has been shown to involve 1D sliding (16). Recently, multiple conformations have been identified by electron microscopy for p53-DNA complexes (25). Further support for a multimode search comes from a single-molecule study of p53 truncation mutants showing that distinct
p53 domains (C-terminal and core) perform sliding and recognition functions (26) (Fig. 1C). The C-terminal domain’s energy landscape is estimated to have $\sigma \approx 0.6 k_B T$, satisfying the requirements for efficient search, while the specifically-binding core domain cannot slide on its own.

Here, we report measurements using single-molecule fluorescence microscopy of p53’s sequence-dependent diffusivity. We observe that p53’s sliding kinetics on λ-phage DNA in the absence of known cognate sites vary by a factor of 1.6 among different regions of DNA. Using a model with both R and S modes and a model with only a single mode, we construct predicted effective energy landscapes for p53 on DNA and demonstrate that the two-mode model but not the one-mode model accounts for the observed variation in diffusivity among regions of the DNA. We further provide evidence that the two identical homodimers making up biologically active tetrameric p53 can bind DNA in dissimilar modes (i.e., “hemi-specifically”). Such binding has been observed for other DBPs binding to oligonucleotides containing “half-sites” (22). Our analysis of p53’s sequence-dependent sliding kinetics reveals that hemi-specific binding is widespread in p53-DNA interactions.

**Results**

To assess whether p53’s diffusivity depends on its position on λ-phage DNA, we recorded trajectories of fluorescently labeled single p53 molecules on DNA that was tethered to the surface of a flow cell and stretched by shear flow, using total internal reflection fluorescence microscopy (Fig. 2). We mapped trajectories to the contour of DNA (Fig. 3A) and made maximum likelihood estimates of diffusion coefficients, $D$, of p53 particles, while accounting for drift from buffer flow and position-dependent DNA fluctuations (Data analysis in Methods). We found that DNA fluctuations cannot account for observed particle diffusivity: The square of the central 95% of the range of the p53 particles exceeds the amplitude of the square displacement of probes covalently attached to the DNA in the limit of long time windows, $\Delta t$, by 1–3 orders of magnitude (Fig. 3B). Each p53 trajectory’s $D$, along with the particle’s range covered on the DNA, is shown in Fig. 3C.

We observed that different regions of the λ-phage DNA correspond to different diffusion coefficients. We determined an aggregate experimental diffusion coefficient, $D_{\text{exp}}$, for each segment by assigning every midpoint of each particle trajectory displacement to a position on the DNA, binning the contour of the DNA into approximately 3-kb segments, and calculating the mean $D$ within each segment (Fig. 3A, C, and D). Error bars in Fig. 3D are errors of estimated $D_{\text{exp}}$ calculated as standard deviation in $D$ from 1,000 bootstrap resamples of the particles in each segment (SI Text, Significance and Consistency of Experimental Results). We found that segments’ aggregate $D$ spanned a factor of approximately 1.6, with 11 of the 36 pairs of segments differing in $D$ significantly at $\alpha = 0.05$ and 6 of the pairs at $\alpha = 0.01$ (SI Text, Significance and Consistency of Experimental Results). Plots of the mean-square displacement as a function of $\Delta t$ for particles in selected segments are shown in Fig. 3E.

Next, we tested whether this variation in $D_{\text{exp}}$ could be explained by sequence-specific binding in R mode. Because λ-phage DNA contains many sites that resemble half- and full-sites of p53, we expected p53 could bind these sites and thus slow down sliding. To this end, we developed a model of an effective two-mode landscape experienced by tetrameric p53 on DNA. Experimental studies have demonstrated that p53, a dimer of dimers with a response element (RE) of 20 bp, binds with one of its dimers to a 10-bp half-site with greater affinity than to random DNA (24, 27). Accordingly, we posited that each dimer could bind a position on DNA in R mode with an energy depending on the sequence, $E_{\text{seq}}(x)$, or in S mode with constant energy $E_0$, while the other dimer could bind in a similar (fully specific or fully nonspecific) or dissimilar mode (hemi-specific) (Prediction of diffusion coefficients in Methods). A cooperation term $\epsilon$ accounts for additional binding energy when both dimers bind in R mode.

We built a sequence-specific landscape $E_{\text{seq}}(x)$ using a position weight matrix (PWM) for a single dimer, based on known p53 REs (28) (Fig. 4A). The PWM was robust to the literature source of REs (SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients). Then, an effective binding energy for the tetramer, $U(x)$, was calculated over all positions according to the two-mode model (Eq. 4 in Methods and SI Text, Eqs. S15–S18), and also according to a single-mode model, giving rise to respective energy landscapes (Fig. 4C and E). The calculation of $U(x)$ allows variable spacing between the 10-bp sequences bound by the two dimers. We identified sites of λ DNA that scored as well as some the weaker known p53 REs, but are not known to be in vivo targets of p53 (Fig. 4B). The PWM was scaled to fit experimentally measured dissociation constants of p53 and oligonucleotides.
containing full-sites, half-sites, and random sequences (24). The difference between $E_S$ and the mean of $E_{R\langle x \rangle}$ was set so that the free-energy difference between specific and non-specific binding for our landscape would match that for typical eukaryotic TFs (29). From the computed landscape, we predicted each segment’s reduction in diffusivity relative to a flat $S$ landscape, $D/D_0$ (Methods and SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients). Areas with more/deep energy wells were found, as expected, to correspond to a reduced diffusivity of particles in the landscape.

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these areas (Fig. 5A). We demonstrate that $D/D_0$ is ratio of the time $t_s$ a protein spends sliding in S mode to the total time the protein spend on the landscape, $t_{total}$:

$$\frac{D}{D_0} = \left< \frac{t_s}{t_{total}} \right> = \frac{n \exp(-2E_S)}{\sum_j \exp(-U(x))}$$

[1]

This result is based on the assumption that traps are isolated or that the protein does not slide in R mode.

We compared $D_{\text{expt}}$ with $D/D_0$ over the segments and found the experimental and predicted diffusion coefficients to correlate strongly ($r = 0.81$, $p = 0.008$) (Fig. 5B, black and red bars). To assess the significance of this correlation, $r_{\text{expt}}$, we constructed 500 random permutations of $E(x)$, computed $U(x)$ and $D/D_0$ for each of them, and determined the resulting $r_{\text{expt}}$ between predicted $D/D_0$ and experimental $D_{\text{expt}}$ over the segments (example in Fig. 5C and D). The correlation between predicted $D/D_0$ and $D_{\text{expt}}$ exceeded that between control $D/D_0$ and $D_{\text{expt}}$ for all but 4 of the 500 control landscapes ($p = 0.008$). The observed strong and significant correlation demonstrates that a two-mode sequence-specific landscape can explain the observed positional variability of p53’s diffusion coefficient. Summary statistics of the segments’ $D_{\text{expt}}$ are given in Fig. 5E.

We also compared the ruggedness of effective landscape $U(x)$, formed from an R and an S landscape, with earlier experiments and with theoretical requirements. Satisfyingly, the global ruggedness $\sigma$ of the two-mode landscape is 0.51 $k_BT$, which lies below the theoretical upper limit for efficient search, $\sim 1.5 k_BT$, and falls within the uncertainty for the aggregate $\sigma$, $0.84 \pm 0.40 k_BT$ obtained for p53 earlier (2). In contrast, the landscape without a nonspecific binding mode has $\sigma = 3.5 k_BT$, which is too great on theoretical grounds for efficient sliding and moreover is incompatible with observed diffusion coefficients. Furthermore, the diffusivity $D/D_0$ computed for the one-mode landscape shows no significant correlation with $D_{\text{expt}}$ ($r = 0.51$, $p = 0.11$), ruling out the one-mode model.

Experimental observations of dimeric p53 binding to half-sites (27) prompted us to explore hemi-specific binding by tetrameric p53. The two-mode model discussed thus far does not require that the two dimers making up the tetramer bind in the same mode. This enhances p53’s affinity even to those half-sites that are flanked by sequences that would be unfavorable to bind in R mode (Fig. 4D). When we eliminated from our model the hemi-specific mode—represented by the two middle terms of Eq. 4, the correlation between segments’ $D_{\text{expt}}$ and predicted $D/D_0$ decreased ($r_{\text{hemi}} = 0.72$ versus $r = 0.81$). The fraction of the sequence-specificity of p53’s diffusion coefficient that owes to full-sites is thus approximately ($r_{\text{hemi}}^2$)/$r^2 = 0.78$, and the fraction that owes to half-sites is approximately 0.22.

As a further test of the two-mode model, we performed Gillespie simulations (30) of a protein undergoing a random walk on the two-mode landscape. Drift from buffer flow and noise

Fig. 5. Comparison of theory, simulations, and experiment. (A) (Left) Predicted potential wells denoted by red (full-sites) and blue (half-sites) bars, with height of bars proportional to predicted effective energy, $U(x)$. Gray bars are a histogram of observed occupancy of all particles within the segment, with bin widths equal to one-twentieth the segment width. (B) Estimated $D$ for experimental (black bars, same as in Fig. 3D) and predicted $D/D_0$ (red bars) for the predicted landscape, over segments along $\lambda$ DNA. $D/D_0$ is scaled to match $D_{\text{expt}}$’s mean and coefficient of variation. Green trace is the percent error in predicted $D/D_0$ normalized by the mean $D/D_0$, relative to $D_{\text{expt}}$ normalized by the mean $D_{\text{expt}}$. (C) Scatter plot of $D_{\text{expt}}$ versus $D/D_0$ for all segments. Red circles correspond to values for the predicted landscape based on the two-mode model; cyan x’s correspond to values for the control landscape whose correlation with $D_{\text{expt}}$ was the median from among the 500 control landscapes. (D) Black bars are identical to those in B. Cyan bars correspond to $D/D_0$ of the control landscape that produced the cyan x’s in C. (E) Summary statistics of experimental data. For each segment, we show the number of particles contributing to the segment’s $D_{\text{expt}}$, the number of displacements contributing likewise, the estimates of $D_{\text{expt}}$ as determined in Data analysis in Methods, and the standard deviation of particle’s $D$ weighted by the number of displacements contributed by that particle. (F) Correlation coefficients and $p$-values.

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from DNA fluctuations were added to the simulated data, which we analyzed identically to the data for experimental $D$s. The simulated and experimental diffusion coefficients across the segments correlate strongly ($r = 0.834$, $p = 0.004$) (Fig. 5F). Simulations on the same 500 control landscapes described above were used to determine statistical significance, and thus provide similar validation as do analytical results of the sequence-specific sliding of p53 by the mechanism of two modes of interaction with DNA.

**Discussion**

We previously proposed a two-mode model of protein–DNA interaction that allows for fast search and specific binding (17, 18). Our earlier single-molecule measurements of p53 sliding on DNA revealed that the protein slides with sufficiently low friction to satisfy the model’s requirements for efficient search (2). The present study shows that p53 can read the sequence of the DNA on which it is sliding, which is essential for sliding to be functional in accelerating target localization. Our data further suggest that the protein reads, in addition to canonical and near-canonical 20-bp full-sites, half-sites of 10 bp. This conclusion agrees with recent studies showing a role for hemi-specific binding in transcriptional activation at high p53 expression levels (31).

Our results indicate that hemi-specific binding is a general phenomenon of p53-DNA interactions, and not limited to a few known half-site REs. In addition to transcriptional activation, we conjecture that hemi-specific binding might serve to titrate p53 or bias the preactivation distribution of p53 on DNA. This latter function especially is suggested by clustering of degenerate p53 REs around canonical REs, which has also been found for other mammalian TFs (32). Odd numbers of half-sites have been found in many p53 binding sites (33, 34); hemi-specific binding would allow finer tuning of transcriptional activation of p53’s targets.

Our model of p53-RE binding energy is based on a PWM approximation that was shown to be sound for the four eukaryotic TFs studied (29) as well as for p53 in the case of good full-sites (35), but it omits some observed peculiarities of p53 REs such as gaps within half-sites (33), stronger conservation within a full-site of the first half-site than the second (33), and transcriptional activation from three-quarter sites (36). Accounting for these complexities might yield a stronger correlation between predicted and experimental $D$, at the expense of model simplicity.

The two-mode model can be generalized to include transition states or a reaction coordinate of the conformational transition in the protein–DNA complex (17, 18). Molecular dynamics studies of TF–DNA association indeed show a range of conformations (37). Our estimate of $D/D_0$ is equivalent to the ratio of partition functions of a flat landscape and the predicted “golf-course” landscape. As such, it is independent of the transition rates between R and S modes, since holding the binding energy of the protein in R mode and in S mode at a given position constant requires the R-to-S and S-to-R rates to vary by the same constant factor. On sufficiently long timescales of sliding, a visit to the R state that lasts $n$ times longer will happen $n$ times less frequently. And although our multiple landscapes are motivated by our prior work on the global protein–DNA search, they are compatible with other such treatments as well (38, 39).

While enough sites on the λ genome have sufficient affinity for p53 to measurably affect its sliding kinetics, in future work, we intend to add known p53 REs to λ and other DNA (SI Text, Control for Specific Binding) to observe stronger sequence-dependent effects, and to answer questions such as whether p53 can slide over a target site without recognizing (binding stably to) it.

We report here the observation of sequence-dependent 1D diffusional kinetics of p53 on DNA. We offer additional experimental support for the importance of 1D diffusion in the kinetics of transcriptional regulation and protein–DNA recognition. With p53 at least, a full understanding of how its complex promoter architecture functions in transcriptional regulation requires consideration of moves by the protein on DNA even after it has found its cognate site and the ability of the protein to recognize both full- and half-sites while undergoing those moves. Evidence for a multimode model of p53’s binding to DNA suggests that the protein’s function may be disrupted not only by the comparatively well-studied mutations in cognate-site binding residues, but also by mutations that affect its nonspecific interaction with DNA or its ability to transition between specific and nonspecific modes, with potential importance for human health.

**Methods**

**Materials and Data Acquisition.** The optical setup, DNA constructs, labeled p53, and flow cells (Fig. 2A) were as described previously (2), excepting that the protein was labeled with AlexaFluor 555 (Invitrogen) and illuminated by the 532 nm line of a Nd:YAG laser, and that fiduciary beads were used to align p53 movies and DNA movies (SI Text, Materials and Data Acquisition).

**Data Analysis.** Protein molecules were assigned to individual DNA molecules, and their trajectories were recorded using scripts written in MATLAB (MathWorks). Positions of the p53 molecules in space along the DNA image were mapped to positions on the contour of the DNA using Brownian dynamics simulations (SI Text, Data Analysis). Fig. 2C shows three sample trajectories.

We determined a diffusion coefficient $D$ for every trajectory recorded except for those of particles stuck to the flow-cell surface (SI Text, Materials and Data Acquisition). We used maximum likelihood estimation, correcting for biased drift from buffer flow as well as for fluctuation in the λ-phae DNA on which p53 diffused. We found $D$ for a particle to be estimated by

$$D = \frac{1}{2} \left( \frac{1}{n} \sum_{i=1}^{n} \left( \frac{\Delta x_i - v \Delta t}{\Delta t} \right)^2 - \frac{1}{n} \sum_{i=1}^{n} \left( \frac{\Delta x_i^2 + \Delta x_i^2}{\Delta t} \right) \right).$$

where $v$ is the drift velocity (SI Text, Eq. S3 in Data Analysis).

An N-frame trajectory contains $(N−1)/N−2)/2 \equiv n$ displacements. The ith observed displacements in space and in time are, respectively, $\Delta x_i$ and $\Delta t_i$. The sum in $D$ is over time windows $\Delta t$ ranging from the camera frame rate, 30 ms, to 2 s. The quantity under the sum is the mean squared displacement of the DNA itself owing to Brownian fluctuations, $(\Delta x_i^2 + \Delta x_i^2)/\Delta t_i$, on a timescale of $\Delta t$, as calculated from measurements of quantum dots covalently attached to the DNA (SI Text, Interpolations of DNA-Fluctuation Variance and Distributions), divided by $\Delta t_i$ and weighted by the number of displacements with a corresponding $\Delta t_i$. The first sum represents the apparent diffusion coefficient of p53, corrected for drift. $D_{null}$ for a segment is the average of all particles in the segment, weighted by the number of displacements contributed.

**Prediction of Diffusion Coefficients.** In its most basic form, our two-mode model posits a recognition (R) mode in which a protein’s binding energy, $E_R$, is dependent on its position on DNA, $x$, and a search (S) mode in which its binding energy, $E_S$, is constant.

To determine $E_R(x)$, we scored the λ genome using position weight matrices ($PWM_M$) of p53 half-sites derived from a catalogue of p53 binding sites (28) (Fig. 4A). We assume that differences between scores are proportional to differences of binding half-site energies:

$$E_R(x) - E_S = c \cdot (PWM_M(x) - PWM_M_S),$$

where $PWM_M(x)$ is the score for position $x$, and $PWM_M_S$ is the score corresponding to binding energy in the $S$ mode, and $c$ is a conversion factor between score and energy (in kcal/mol). Since p53 is a dimer of dimers with each dimer able to bind independently to a 10-bp half-site (27). For binding in $R$ mode, then, the left dimer binds with energy $E_R(x)$ and the right dimer binds with energy $E_R(x + \Delta)$. The statistical weight $w(x)$ of a site $x$ is thus the sum of the Boltzmann factors corresponding to each of the four modes, and the negative logarithm of $w(x)$ is the effective binding energy of p53 to λ DNA at position $x$. 

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As described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*, we found that $D/D_0$ in a segment should equal the mean fraction of time a particle in that segment spends sliding, $t_s/t_{total}$, which in turn equals the reciprocal of the average statistical weight (equivalent to $\exp(-U(x))$) over all sites in the segment of Eq. 4. We assume that microscopic step rates to positions $x - 1$ and $x + 1$ are equal.

Once we computed $D/D_0$ for each segment, we made a correction to account for uncertainty in the assignment of experimental displacements to segments owing to DNA fluctuations, described in *SI Text, Interpolations of DNA-Fluctuation Variance and Distributions*. We then assessed the quality of our predicted diffusion coefficients (beta07) with a surrogate Pearson's correlation coefficient $r_{exp}$ between experimental diffusion coefficients $D_{exp}$ and predicted $D/D_0$ over the segments, and determined statistical significance of the correlation using a permutation test described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*.