Review

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The "Monkey-Bar" Mechanism for Searching for the DNA Target Site: The Molecular Determinants

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Abstract: DNA recognition by DNA-binding proteins, which is a pivotal event in most gene regulatory processes, is often preceded by an extensive search for the correct site. A facilitated diffusion process, in which a DBP combines 3D diffusion in solution with 1D sliding along DNA, has been suggested to explain how proteins can locate their target sites on DNA much faster than predicted by 3D diffusion alone. One of the key mechanisms in the localization of the target by a DNA-binding protein is intersegment transfer in which the protein forms a bridged intermediate between two distant DNA regions. This jumping mechanism is more enhanced when the DNA-binding protein is asymmetric in its structure or its dynamics. We suggest that asymmetry supports the "monkey bar" mechanism, in which different domains of the protein interact with different DNA regions. In this minireview, we discuss how the molecular architectures of the proteins and DNA may modulate the efficiency of monkey bar dynamics.

Keywords: DNA–protein interactions · DNA search · facilitated diffusion · monkey-bar mechanism

Introduction

One of the most fundamental aspects of protein–DNA recognition is how proteins find their target sites so rapidly, given the millions of competing nonspecific sites on DNA and the packed structure of DNA in the cell. The association rate of a transcription factor with its operator on DNA can be two orders of magnitude faster than the maximal rate of a bimolecular reaction controlled by 3D diffusion.[1] Following the study of Berg et al.,[2] it has become widely accepted that the search by a protein for its target DNA sequence is achieved through facilitated diffusion comprising four different mechanisms: 1D sliding, hopping (also known as correlated transfer), 3D search, and intersegment transfer.

The notion of a facilitated target-search mechanism, whereby diffusion occurs in a lower dimensionality space,[1,3–7] was originally developed from both theoretical and experimental perspectives,[2,8] and was later supported by various bulk experiments that provided indirect support for the concept of linear diffusion on DNA and for the importance of the combination of 1D and 3D diffusion to speed up protein–DNA binding.[1,9–11] Furthermore, in recent years, several studies at the single-molecule level have visualized 1D diffusion of a protein along DNA.[12] For example, sliding along DNA has been directly observed for RNA polymerase,[13] *lac* repressor,[14,15] DNA repairs,[16] and the p53 transcription factor.[11]

The biophysical characterization of facilitated diffusion was studied by various experimental techniques,[9,12,17–20] as well as by computational[21–24] and theoretical approaches.[25–29] The molecular and dynamic nature of protein searching for DNA was studied using molecular dynamic simulations,[30] particularly using reduced models[31–33] that allow sampling of long timescale processes, such as sliding, hopping, 3D diffusion, and intersegment transfer. We modeled DNA as having three beads per nucleotide, representing phosphate, sugar, and base. Each bead was located at the geometric center of the group it represented. A negative point charge was assigned to beads that represented the DNA phosphate groups. The nonspecific interactions between the protein and DNA was modeled by electrostatic interactions that were modeled by the Debye–Huckel potential.

Experimental studies, together with computational studies of the structural and dynamic features of proteins on a single DNA molecule using a coarse-grained model, showed that sliding was a stochastic motion during which the proteins followed a helical path along the major groove.[9,17,23,34] During sliding, the protein makes use of the interface that defines its specific binding to DNA. The spiral motion of the protein along the phosphate–sugar rails was hypothesized about 30 years ago by Schurr[35] and recently was confirmed elegantly by a series of NMR spectroscopy studies of HoxD9 protein by the Clore group.[17,36,37] While the sliding motion is unambigu-

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ous and distinctive, hopping dynamics are less characterized. Often hopping is described as consisting of dissociation events of short lifetime, in which the protein reassociates with DNA at a point in the vicinity of the dissociation position, whereas in the 3D search the protein reassociates at a point uncorrelated with the original location of the dissociation event. During hopping, as in sliding, the protein faces the DNA, but its orientation relative to DNA is more diverse because it is not placed at the major groove.[23] Not being helically constrained, linear diffusion along the DNA is therefore faster during hopping than during sliding, when the dynamics are restricted by the backbone rails.

Dana Vuzman received her MSc in 2008 and her PhD in 2012 from the Weizmann Institute of Science, Israel. During her MSC she worked with Prof. David Milstein on synthesis, reactivity, and characterization of organometallic pincer complexes. In her doctoral studies she worked with Prof. Yaakov Levy in the field of computational biophysics on the characteristics and effects of disordered regions of proteins on DNA search. She then joined the group of Prof. Shamil Sunyaev at Harvard Medical School (HMS) and Brigham and Women's Hospital (BWH), USA. In her postdoctoral studies, she used familial DNA sequencing data to map genes and watch evolutionary processes in real time. In 2014, she joined the Personal Genomics consultation Service (PGCS) in HMS and BWH as an associate director. In PGCS she leads the collaborations with Clinical Geneticists, Computational biologists and other specialists at the institution, throughout the Harvard Medical affiliates, and throughout the world, to find new genetic causes for rare genetic phenotypes of BWH patients.

Yaakov Levy is an associate Professor at the Dept. of Structural Biology at the Weizmann Institute of Science. He received his PhD in chemical physics from Tel-Aviv University, Israel, in 2002, where he worked with Profs. Joshua Jortner and Oren Becker on energy landscape characterization of polypeptides. He then joined the labs of Profs. José Onuchic and Peter Wolynes at the University of California at San Diego and the Center for Theoretical Biological Physics. In his postdoctoral studies, he focused on understanding the principles of biological self-organization processes using minimalist models. In 2006, he joined the Dept. of Structural Biology at the Weizmann Institute of Science where he studies various problems in molecular biophysics using theoretical and computational tools. In particular, his group is focused on the effect of post-translational modifications on the biophysics of protein folding and the molecular determinants that control the speed and efficiency of DNA search.

The existence of intersegment transfer has been confirmed by a number of in vitro experiments for various proteins, such as homeodomain HoxD9[36] and multidomain Oct-1.[18,38] The latter can adsorb two segments of DNA, which allows Oct-1 to transiently form a double-bound state with two DNA fragments. This double-bound state breaks up faster than the dissociation of protein to the bulk from the fully formed protein–DNA complex, and the protein has a chance to be transferred to newly adsorbed DNA. It has been suggested that intersegment transfer significantly accelerates the search for a specific target site on DNA under conditions where the protein is adsorbed onto the DNA most of the time, as it is in vivo.[39–41]

In addition to the characterization of the protein linear diffusion on DNA (e.g., the value of the diffusion coefficient and the existence of jumping or hopping events) and the dynamics of intersegment transfer obtained from ensemble and single-molecule measurements, some aspects of the molecular characteristics of protein search on DNA have been quantified in recent years. Describing the molecular aspects of DNA search is essential for understanding the interplay between the structural architecture of proteins or DNA and the efficiency of the DNA search. Clearly, the dimensions and structural details of the recognition region on the proteins that directly interacts with the DNA in a specific binding interaction affect the mode and efficiency of the DNA search when the protein interacts with the DNA nonspecifically. It was shown that the degree of overlap between the electrostatic patch that is in use in nonspecific interactions with DNA and the patch involved in hydrogen bonding with the specific DNA dictates the sliding speed and the rate of conversion from nonspecific to specific binding.[42,43] In addition to the structural characteristics of the binding sites, the overall molecular architecture of the protein may affect the DNA search for its target site. For example, the oligomeric state of the protein can affect its motion along DNA. Homodimeric proteins may slide very differently than their monomeric variants.[44] The interactions of tetrameric proteins, such as p53, with nonspecific DNA contrast sharply with those of mono- or dimeric p53. Compared with single-domain proteins, proteins composed of several domains may also show unique biophysical characteristics when interacting with DNA.

In intersegment transfer, direct relocation from one nonspecific DNA to another occurs without accumulation of free protein. We have shown that intersegment transfer is promoted when the protein is composed of at least two domains. The two domains can be globular proteins connected by a flexible linker or a protein with a less structured subdomain (e.g., a protein with a disordered tail). A tailed protein or a two-domain protein translocates on DNA molecules through an intermediate in which the recognition region on one of the protein domains is adsorbed onto one DNA fragment, while the recognition

region on the other protein domain is adsorbed onto a second DNA fragment. We refer to this dynamic as the "monkey-bar" mechanism because it resembles the motion of children as they swing along monkey bars in a playground. Similarly to the way a child transfers one hand at a time when swinging from bar to bar, the proteins cross from one DNA molecule or segment to another by transferring first a single domain, followed, after a certain lag time, by the transition of the other domain (or subdomain, e.g., a disordered tail). In this brachiating dynamics (which may also be described as following the fly-casting mechanism[45–48]), the disordered tail or a low-affinity globular domain reaches relatively distant DNA regions that lie beyond those near the other domain of the protein, and therefore, fly-casting suggests an increase in the kinetic specificity of the search.[46]

The monkey-bar mechanism requires not only that the proteins will comprise at least two structural elements (two domains or a domain and a disordered tail), but there will also be a degree of asymmetry in the dynamics of the constituent domains and in their affinity to the DNA. In addition to the properties of the protein, the molecular details of the DNA may also control the usage of the monkey-bar mechanism. The molecular details of the monkey-bar mechanism are discussed below.

The Role of Protein Asymmetry in Monkey-Bar Dynamics: Multidomain Proteins

Proteins composed of multiple domains are very common, especially in eukaryotic genomes.[49,50] In many cases, these domains lack any interface and can cooperate only through a disordered, flexible linker. Flexible linkers are crucial to several types of functions in protein–DNA recognition.[51] The linker can attach unfolded domains and keep the protein as a folded dimer that can bind to DNA, such as in the designed single-chain Arc repressor.[51,52] In other cases, the disordered linker can tether the DNA-binding domain with a dimerization domain as in the λ repressor,[51,53] or with a tetramerization domain, as in p53,[54] and enhance the binding affinity.[51] The disordered linker was shown to be crucial for DNA binding and recognition by Oct-1[55] and for the human replication protein A (RPA).[56] We focus on linked domains, such as those found in the Pax6,[57] Oct-1,[7,58] and p53[59] transcription factors, in which the disordered linkers that connect between the different DNA-binding domains increase their affinities to DNA over those of the isolated domains.

The molecular mechanisms of DNA search by transcription factors consisting of two DNA-binding-domains demonstrated that, when Pax6 and Oct-1 engaged in non-specific binding, their individual domains exhibited significantly different affinities to DNA;[57] this finding is supported by experimental results.[58] Tethering the two domains through a flexible linker increases their affinity to the DNA,[51] resulting in a higher propensity for sliding along DNA, which is more significant for the domain with the weaker DNA-binding affinity.[57] The domain that binds DNA more tightly anchors the multidomain protein to DNA and, through the linker, increases the local concentration of the weak DNA-binding domain at the expense of slower linear diffusion than that exhibited by the isolated domains.

Computational[57] and NMR spectroscopy[18,38,60] studies showed that multidomain transcription factor exchange occurred between two DNA molecules via a bridged intermediate, in which each domain was attached to a different DNA segment, suggesting the monkey-bar mechanism[61] (Figure 1). Intersegmental transfer is facilitated when the individual domains are tethered by a flexible linker. The electrostatic affinity of each domain to DNA significantly affects the number of jumping events. Greater facilitation of the jumping search mode is achieved when one domain has a higher affinity to DNA and acts as a strong anchor, while the complementary domain has a lower DNA binding affinity and serves as an explorer for binding sites on adjacent DNA molecules, as shown for the Pax-6[57] and Oct-1[18,38,57] transcription factors (Figure 1).

The disordered linker mediates cross-talk between two domains when there is no physical interface between

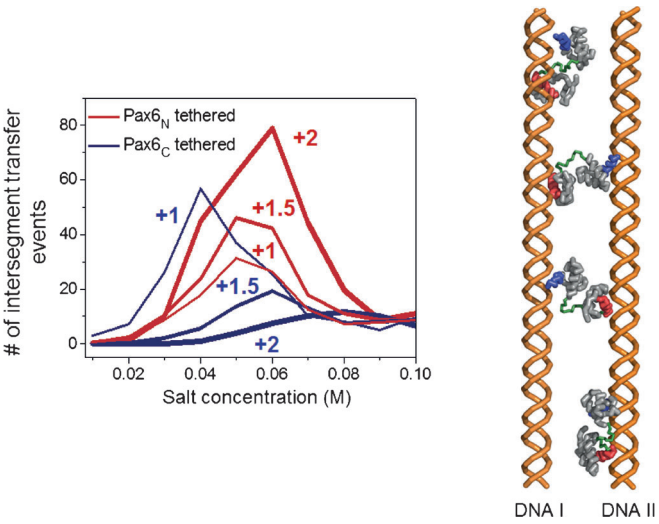

Figure 1. Monkey-bar dynamics between two parallel DNA molecules by the two-domain transcription factor. Left: Asymmetry between the affinity of the two constituent domains of Pax6 increases the number of monkey-bar brachiations. The panel shows the number of intersegment transfer events performed by tethered Pax6 variants (WT $Pax6^{+1}$ (thin line), $Pax6^{+1.5}$ (medium line), and $Pax6^{+2}$ (thick line)). In these variants, the magnitude of the charges in the C-terminal domain of Pax6 ($Pax6_C$) was changed, while the N-domain ($Pax6_N$) remained unchanged. A higher affinity of the C-domain to DNA results in a greater number of intersegment transfer events performed by the N-domain in the tethered variants. Right: Pictorial representation of four frames from the Pax6 dynamics on two parallel DNAs.

them. Tethering by the linker can lead to a significant improvement in the search efficiency, which strongly depends on the relative affinities of the domains to DNA. A comparison of the electrostatic contributions to the protein–DNA binding energy that are made by the individual domains comprising several multidomain proteins reveals that tethered domains tend to have different DNA-binding affinities, which may imply that both specific and nonspecific DNA binding has biological significance.[57] The efficient DNA search observed for multidomain proteins with different affinities to DNA and the polarized DNA-binding affinities found for multidomain proteins suggest that there is an evolutionary drift toward tethering of domains with different affinities to DNA by a flexible linker.

More evidence for the role of asymmetry in multidomain proteins in promoting the monkey-bar mechanism was observed for the zinc finger protein Egr-1, which is composed of three fingers (ZF1–3). It was shown by NMR spectroscopy measurements and coarse-grained molecular dynamics simulations that ZF1 was more dynamic than the two other fingers.[62,63] The high degree of ZF1's dynamics in the nonspecific complex can be attributed to 1) weaker electrostatic interactions with DNA and 2) weaker interdomain interactions with ZF2. Electrostatic binding free energy calculations suggested that among the three zinc finger domains, ZF1 was the weakest DNA binder with the lowest absolute value of electrostatic binding free energy (-1.6 kcal/mol, as opposed to -3.2 kcal/mol for ZF3). This can qualitatively be attributed to the smaller net charge of ZF1 ($+1e$) compared with ZF2 ($+4e$) and ZF3 ($+4e$). ZF1's weak interdomain interaction with ZF2 can be another determinant. It is possible that other factors (e.g., the strength of the interface between neighboring fingers) also support the higher dynamics of ZF1. The simulations show that the intersegment transfer events of Egr-1 occurred via an intermediate, in which an Egr-1 molecule transiently bridges two DNA molecules, which follows the monkey-bar mechanism. For the wild-type protein, the bridging events occurred most frequently by ZF1 capturing the other DNA molecule, but mutations that increased the nonspecific affinity of ZF1 to DNA reduced the efficiency of the monkey-bar dynamics by making the dynamics more symmetrical; it was also shown by kinetic NMR spectroscopy that the rate of locating the target site was slower. The study of Egr-1 suggests that its asymmetric search mode of DNA (i.e., ZF1 is more mobile and less attracted to DNA than ZF2 and ZF3) is suitable for intersegment transfer that involves the transient bridging of two DNA sites. We note that the asymmetric dynamics of this symmetric (repeat) protein may not be simply governed by the overall net charge of each domain, but also on the absolute number of charges and their locations, which may both affect the electrostatic potential.

The Role of Protein Asymmetry in Monkey-Bar Dynamics: Disordered Tails

We have previously shown that disordered tails are more common in DNA-binding proteins than in non-DNA-binding proteins.[57,64] The tails of DNA-binding proteins are longer than those of other proteins ($\approx$60% of the tails of DNA-binding proteins are longer than 5 residues, whereas only 30% of non-DNA-binding proteins have tails longer than 5 residues). The disordered tails of DNA-binding proteins have a larger net positive charge and these charges tend to be much more clustered in the tails of DNA-binding proteins than in the tails of non-DNA binding proteins. We note that the composition of positively charged residues and their degree of clustering in the structured parts of DNA-binding proteins and non-DNA binding proteins are very similar.[64]

A recent study based on coarse-grained molecular dynamic simulations of three homeodomain proteins with different tail lengths and net charges, in which the interactions between proteins and DNA are governed solely by electrostatic forces, demonstrated the role of the disordered tail in facilitating DNA search,[61] in agreement with kinetic NMR spectroscopy studies.[17,37] The presence of an N-tail increases the affinity of the protein to the DNA, and therefore, enhances its sliding propensity at the expense of hopping and 3D diffusion. However, better sliding has its price: the linear diffusion coefficient of the protein moving along DNA is lower, which results in a slower search.[61] The increased use of the sliding search mode accompanied by the decreased use of linear diffusion in the presence of an N-tail is more pronounced for the Antp homeodomain than for the HoxD9 homeodomain, which correlates with the presence of a longer tail and a higher net positive charge for Antp than HoxD9.

Most importantly, the N-tail in homeodomains can assist intersegment transfer, which is known to enhance DNA search when the protein interacts nonspecifically with DNA.[38–41] Direct relocation from one nonspecific DNA sequence to another occurs without accumulation of free protein during the intersegment transfer of transcription factors.[37,57,61,64] Thus, intersegment transfer is facilitated by the disordered tail. Tail length can significantly affect the propensity to engage in intersegmental transfer, such that more jumping events are seen for the Antp homeodomain, the tail of which is longer and more positively charged (10 residues and net charge of $+4$), than for the HoxD9 homeodomain, which has a shorter and less positively charged tail (9 residues and net charge of $+2$). The three homeodomain proteins, HoxD9, Antp, and NK-2 (see ref. [61]) search DNA quite differently, although they have very similar globular regions. The presence of an N-tail in these homeodomains modulates the characteristics of the DNA search. Although the efficiency of the DNA search is correlated with tail length and

net charge, the linkage is more complex than that. A comparison of the tail sequences of these three homeodomains reveals that, while the lengths and net charges are different for each, the positive residues are clustered into positive segments in all of them. Simulations of more than 600 homeodomain variants with different net charges and positive charge distributions on the tails showed that the intersegment transfer search mode is most populated for moderately charged segments on the tail and for positive charges that are clustered together in the middle of the tail or toward its N terminus[64] (Figure 2).

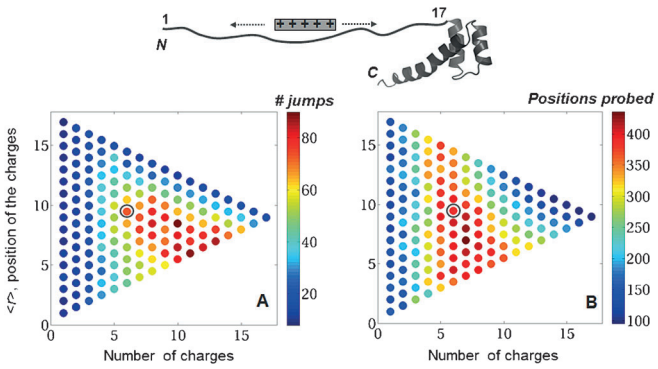

Figure 2. Monkey-bar dynamics between two parallel DNA molecules by a transcription factor with disordered tails. The effect of the length and location of positively charged segments in the disordered N-tail on the properties of DNA search by the homeodomain NK2. The influence of the effective charge and the average position, $<r>$, of the charged segment on the tail on the number of intersegment transfer event (left) and the number of position probed by sliding (right) performed by the 153 variants of the NK-2 homeodomain between two DNA fragments separated by 60 Å at salt concentration of 0.07 M. The length of the positive segment was varied from 1–17 consecutive charged residues and it was positioned at all possible positions along the 17 positions of the tail (lower $<r>$ values mean that the charged residues are centered closer to the N terminus). The gray circle indicates the effective charge and $<r>$ values of the N-tail of wild-type NK-2, suggesting that its sequence was evolved to promote the monkey-bar mechanism. The sequence of the tail of NK-2 is ASDGLPNKKRKRRVLFF.

The tailed proteins might be viewed as multidomain proteins, in which the disordered tail serves as a subdomain. In both cases, the composition of the proteins of several domains together with the asymmetry (in their dynamics and binding affinity to DNA) is essential for promoting intersegment transfer through the monkey-bar mechanism.

Transcription factor p53 provides another example of the importance of disordered tails in the interactions between proteins and DNA.[65,66] The C-tails of p53 are positively charged, and thus, strongly interact with DNA and can affect sliding features very significantly. Recent single-molecule experiments[10] and a coarse-grained simulation study[59] showed that the C-tails of p53 increased its sliding speed along DNA. In the truncated version, the sliding speed of p53 slows significantly. Moreover, the disordered C-tails mediate interactions between the DNA-binding domain (the core domain) and distant DNA regions where tails from two subunits interact with one DNA region, while the other two tails interact with a different DNA.

The Effects of the Relative Orientation of the DNA Molecules on the Monkey-Bar Dynamics

Intersegment transfer was shown to significantly improve DNA search efficiency, depending on the molecular characteristics of the transcription factors.[39,61,67] The ability of a two-domain protein (for example, proteins that are composed of two globular domains connected through a flexible linker or a protein composed of a globular domain and a long disordered tail) may also depend on the molecular properties of the DNA. For example, the distance between the two DNA segments will clearly control the usage of the monkey-bar mechanism.[61] Clearly, two-domain proteins with longer linkers can form a bridged intermediate between two more widely separated DNA sites than two-domain proteins with shorter linkers. The distance between the two DNA segments and the length of the disordered linker (or the disordered tail) are correlated with the ability to jump. Another parameter that may affect the probability to jump using the monkey-bar mechanism is the relative orientation of the two DNA regions.

Herein, we investigated the roles played by the inter-DNA angle and inter-DNA distance in the protein search of DNA. These aspects are addressed by quantifying the molecular characteristics of intersegment transfers undertaken by the NK-2 and Antp homeodomain transcription factors. The search by each homeodomain of two 100 base pair (bp) DNA molecules separated by various distances (40–70 Å) and rotated to various extents (0–360°) relative to each other at a salt concentration of 0.07 M was simulated using a simple computational model[23,61] in which protein–DNA interactions were represented solely by electrostatic forces.

The portions of the search performed using intersegment transfer, during which the globular domain of NK-2 or Antp was on one DNA molecule, while its disordered tail was on the other DNA molecule, are shown in Figure 3. The color of the scatter plots represents the number of the jumps during the simulation time. A short distance of 40 Å between DNA molecules is associated with lower usage of the monkey-bar mechanism for all rotational angles because of higher usage of a bridged intermediate at the junction between the DNA molecules. At a longer inter-DNA distance of 70 Å, electrostatic attraction between the DNA molecules and the DNA binding regions on the protein, namely, the tail and recognition helix, is insufficient to promote jumping through

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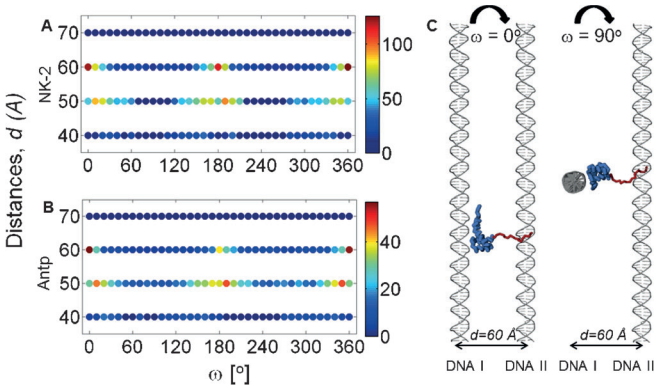

Figure 3. The effect of the rotation angle between the two DNA molecules on the number of monkey-bar brachiations. The influence of distance and angle between two DNA molecules, at salt concentration of 0.07 M, on the jumps search mode for NK-2 (A) and Antp (B) proteins. The color corresponds to the number of jumps during simulation. The distance, *d*, and angle, ω, are altered in the simulations by 10 Å and 10° in the range of 40–70 Å and 0–360°, respectively. C) Graphical representation of the NK-2 tail (red) and globular part (blue) on two DNA molecules. In the two snapshots, the two DNA molecules are separated by 60 Å and the inter-angles are 0 and 90°.

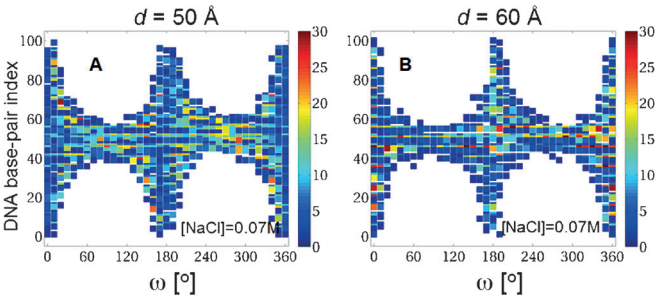

Figure 4. Jump location obtained through the monkey-bar mechanism. The number of jumps is projected along the index of the DNA bp and the angle for Antp protein on two 100 bp DNA molecules at a salt concentration of 0.07 M separated by 50 (A) and 60 Å (B). For the parallel DNA orientation, the jump can start from any region along DNA. When the angle between DNA molecules transforms from parallel to perpendicular orientation, the assembly of jump locations narrows towards the junction between DNA fragments (around bp 50).

a monkey-bar mechanism at all rotation angles. Moderate distances of 50 and 60 Å, which are common in the nucleosome, promote the monkey-bar mechanism for parallel (angles of 0, 180, or 360°) and almost-parallel DNA orientations, where both DNA recognition domains on the protein are within the electrostatic field of the DNA. At an inter-DNA distance of 60 Å, the highest occupation of the intersegment transfer search mode is achieved at perfectly parallel inter-DNA angles of 0, 180, and 360°. By contrast, at 50 Å, many jumps also occur at the almost-parallel inter-DNA rotational angles of 10, 20, 160, 170, 190, 200, 340, and 350° because the tail is long enough to bridge to the other DNA for small rotation of the DNA. Figure 3 shows two snapshots of jumps between two DNA molecules separated by 60 Å and rotated 0 and 90° relative to each other, respectively. Although both homeodomain proteins demonstrate similar occupation of the jump search mode, the total number of jumps performed is higher for NK-2 because of its longer tail with its higher net positive charge and higher clustering index, each of which was demonstrated to promote a more efficient search.[64]

The location of the jumps is highlighted when plotting the number of intersegment transfers as a function of the DNA bps and the orientation angle (Figure 4). When the DNA orientation changes from parallel to perpendicular, jumping tends to occur between the two closest points (i.e., at around bp 50 out of 100 on both molecules), which correspond to the points closest to an imaginary junction formed by extrapolations of the lines of the two DNA molecules. For the perpendicular orientation, the range of DNA bps that promote jumping around the "junction" is larger, at 50 Å compared with 60 Å (Figure 4A and B, respectively), because of the higher electrostatic field exerted by the DNA molecules at the shorter distance. This explains the higher number of jumps observed in Figure 3 at a nonparallel inter-DNA orientation and an inter-DNA distance of 50 Å. The probability to jump is smaller for a separation distance of 60 Å than for 50 Å, and in this case the number of jumps is more sensitive to changes in the rotation angle, ω, between the two DNA molecules.

Figure 3 shows two snapshots of monkey-bar dynamics for parallel (0°) and perpendicular (90°) inter-DNA orientations at an inter-DNA distance of 60 Å. Although the former orientation supports intersegment transfer at any starting point along the original DNA, the latter only allows jumping that originates from the middle of the DNA, where the distance to the other DNA is the shortest. Accordingly, the separation distance between the DNA molecules and the angle between them dictates the distance between two sites on DNA and whether it can be bridged by a given tail.

We have previously shown that the intersegment transfer search mode is highly dependent on salt concentration for DNA molecules oriented in parallel to each other.[61] Herein, we investigated the influence of DNA geometry at different salt concentrations for two DNA fragments separated by 60 Å. The number of jumps performed by NK-2 (Figure 5A) and Antp (Figure 5B) homeodomains as a function of inter-DNA rotation angle at varying salt concentrations (0.05–0.08 M) demonstrates that, in a parallel DNA orientation of around 0, 180, and 360°, the population of the jump search mode is dependent on salt concentration. By contrast, at other DNA orientations, the number of jumps is low at all salt concentrations. The salt effect is more pronounced for the Antp protein, which searches DNA most efficiently at a salt concentration of

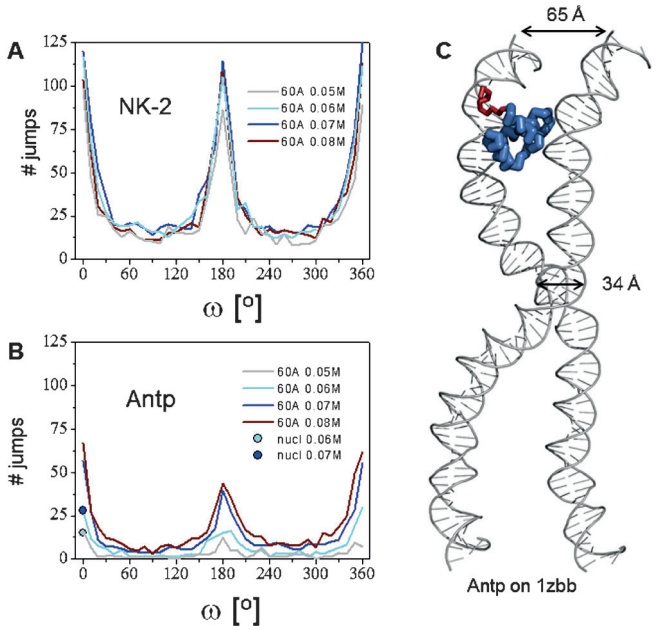

Figure 5. Interplay between the angle between two DNA molecules and salt concentration on the monkey-bar events. Quantitative characteristics of intersegment transfer for NK-2 (A) and Antp (B) at a distance of 60 Å between DNA molecules with altering angles in the range of 0–360° and salt concentrations in the range of 0.05–0.08 M. C) Graphical representation of the Antp tail (red) and globular part (blue) on a nucleosomal 72 bp DNA segment (DNA was derived from PDB ID: 1ZBB).

0.08 M, and least efficiently at 0.05 M, even for parallel DNA orientations (Figure 5B). NK-2 has a tail with charge properties that are optimal for an efficient search,[67] which enables a high number of jumps, even at a low salt concentration of 0.05 M.

To examine the search process on a DNA molecule with biologically relevant geometry, we studied the dynamics of the Antp protein on two double-stranded DNA molecules, each consisting of 72 bps and derived from nucleosomal DNA (PDB ID 1ZBB). The DNA segments were separated by 64 Å at the edges and by 34 Å at the "junction" (Figure 5C). The population of the jump search mode during the simulation at a salt concentration of 0.07 M is comparable to the number of jumps achieved by Antp when searching parallel DNA molecules separated by 60 Å at a salt concentration of 0.06 M.

Conclusions

In this minireview, we discussed how the molecular characteristics of DNA-binding proteins and DNA may control the usage of the monkey-bar mechanism in searching DNA. We illustrated how the molecular details of the proteins, such as their internal flexibility or electrostatic affinity to DNA, could affect this jumping mechanism. In particular, the asymmetry within the protein is central for efficient intersegment transfer through the monkey-bar mechanism. We showed that proteins composed of several domains with different flexibility and DNA affinity were advantageous for fast search. It will be intriguing to investigate in the future how the degree of asymmetry is linked to the function carried out by each protein or by the need to rapidly locate the target site. We also discussed that the molecular properties of the DNA might also affect the usage of the monkey-bar dynamics. We illustrated how the relative orientation (interdistance and -angle) might control the jumping propensity, but it was still to be quantified how the DNA dynamics and flexibility would change the monkey-bar mechanism. This minireview illustrated, through several examples, that, while facilitated diffusion was common to many DNA search processes, the molecular properties of the proteins and DNA might regulate its details, presumably in a way that is linked to the function carried out by each protein–DNA recognition interaction.

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References

[1] S. E. Halford, *Biochem. Soc. Trans.* **2009**, *37*, 343–348.
[2] O. G. Berg, R. B. Winter, P. H. von Hippel, *Biochemistry* **1981**, *20*, 6929–6948.
[3] S. E. Halford, J. F. Marko, *Nucleic Acids Res.* **2004**, *32*, 3040–3052.
[4] M. Slutsky, L. A. Mirny, *Biophys. J.* **2004**, *87*, 4021–4035.
[5] K. V. Klenin, H. Merlitz, J. Langowski, C. X. Wu, *Phys. Rev. Lett.* **2006**, *96*, 018104.
[6] Z. Wunderlich, L. A. Mirny, *Nucleic Acids Res.* **2008**, *36*, 3570–3578.
[7] N. Shimamoto, *J. Biol. Chem.* **1999**, *274*, 15293–15296.
[8] P. H. Vonhippel, O. G. Berg, *J. Biol. Chem.* **1989**, *264*, 675–678.
[9] D. M. Gowers, G. G. Wilson, S. E. Halford *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15883–15888.
[10] A. R. Fersht, A. Tafvizi, F. Huang, L. A. Mirny, A. M. van Oijen, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 563–568.
[11] A. Tafvizi, F. Huang, J. S. Leith, A. R. Fersht, L. A. Mirny, A. M. van Oijen, *Biophys. J.* **2008**, *95*, L1–L3.
[12] J. Gorman, E. C. Greene, *Nat. Struct. Mol. Biol.* **2008**, *15*, 768–774.
[13] C. Bustamante, M. Guthold, X. S. Zhu, G. L. Yang, *J. Biol. Chem.* **1999**, *274*, 16665–16668.
[14] Y. M. Wang, R. H. Austin, E. C. Cox, *Phys. Rev. Lett.* **2006**, *97*, 048302.
[15] J. Elf, G. W. Li, X. S. Xie, *Science* **2007**, *316*, 1191–1194.
[16] A. Graneli, C. C. Yeykal, R. B. Robertson, E. C. Greene, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 1221–1226.

[17] J. Iwahara, G. M. Clore, *Nature* **2006**, *440*, 1227–1230.
[18] Y. Takayama, G. M. Clore, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, E169–E176.
[19] R. H. Porecha, J. T. Stivers, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10791–10796.
[20] J. D. Schonhoft, J. T. Stivers, *Nat. Chem. Biol.* **2012**, *8*, 205–210.
[21] A. Tafvizi, F. Huang, A. R. Fersht, L. A. Mirny, A. M. van Oijen, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 563–568.
[22] A. Marcovitz, Y. Levy, in *Innovations in Biomolecular Modeling and Simulations, Vol. 24*, Royal Society of Cambridge, Cambridge, **2012**, pp. 237–262.
[23] O. Givaty, Y. Levy, *J. Mol. Biol.* **2009**, *385*, 1087–1097.
[24] N. R. Zabet, B. Adryan, *Mol. BioSyst.* **2012**, *8*, 2815–2827.
[25] L. Mirny, M. Slutsky, *J. Phys. A Math. Theor.* **2009**, *42*, 434013.
[26] M. Sheinman, O. Benichou, Y. Kafri, R. Voituriez, *Rep. Prog. Phys.* **2012**, *75*, 026601.
[27] G. Li, O. Berg, J. Elf, *Nat Physics* **2009**, *5*, 294–297.
[28] M. A. Lomholt, B. van den Broek, S. M. Kalisch, G. J. Wuite, R. Metzler, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8204–8208.
[29] A. Kolomeisky, *Phys. Chem. Chem. Phys.* **2011**, *13*, 2088–2095.
[30] E. G. Marklund, A. Mahmutovic, O. G. Berg, P. Hammar, D. van der Spoel, D. Fange, J. Elf, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 19796–19801.
[31] J. K. Noel, P. C. Whitford, K. Y. Sanbonmatsu, J. N. Onuchic, *Nucleic Acids Res.* **2010**, *38*, W657–W661.
[32] R. Li, R. Chen, Y. Wen, P. Ke, S. Cho, *J. Phys. Chem. B* **2013**, *117*, 13451–13456.
[33] P. Anand, A. Schug, W. Wenzel, *FEBS Lett.* **2013**, *587*, 3231–3235.
[34] T. Terakawa, H. Kenzaki, S. Takada, *J. Am. Chem. Soc.* **2012**, *134*, 14555–14562.
[35] J. M. Schurr, *Biophys. Chem.* **1979**, *9*, 413–414.
[36] J. Iwahara, G. M. Clore, *J. Am. Chem. Soc.* **2006**, *128*, 404–405.
[37] J. Iwahara, M. Zweckstetter, G. M. Clore, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15062–15067.
[38] M. Doucleff, G. M. Clore, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13871–13876.
[39] T. Hu, B. Shklovskii, *Phys. Rev. E* **2007**, *76*, 051909.
[40] M. Sheinman, Y. Kafri, *Phys. Biol.* **2009**, *6*, 016003.
[41] C. Loverdo, O. Benichou, R. Voituriez, *Phys. Rev. Lett.* **2009**, *102*.
[42] A. Marcovitz, Y. Levy, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 17957–17962.
[43] A. Marcovitz, Y. Levy, *J. Phys. Chem. B* **2013**, *117*, 13005–13014.
[44] N. Khazanov, A. Marcovitz, Y. Levy, *Biochemistry* **2013**, *52*, 5335–5344.
[45] B. A. Shoemaker, J. J. Portman, P. G. Wolynes, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8868.
[46] E. Trizac, Y. Levy, P. Wolynes, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 2746–2750.
[47] A. G. Turjanski, J. S. Gutkind, R. B. Best, G. Hummer, *PLoS Comput. Biol.* **2008**, *4*, e1000060.
[48] J. Chen, *J. Am. Chem. Soc.* **2009**, *131*, 2088–2089.
[49] S. A. Teichmann, J. Park, C. Chothia, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14658–14663.
[50] G. Apic, J. Gough, S. Teichmann, *J. Mol. Biol.* **2001**, *310*, 311–325.
[51] H. X. Zhou, *Biochemistry* **2001**, *40*, 15069–15073.
[52] M. Sieber, R. K. Allemann, *Nucleic Acids Res.* **2000**, *28*, 2122–2127.
[53] H. X. Zhou, *J. Am. Chem. Soc.* **2001**, *123*, 6730–6731.
[54] A. C. Joerger, A. R. Fersht, *Oncogene* **2007**, *26*, 2226–2242.
[55] H. C. van Leeuwen, M. J. Strating, M. Rensen, W. de Laat, P. C. van der Vliet, *EMBO J.* **1997**, *16*, 2043–2053.
[56] P. D. Vise, B. Baral, A. J. Latos, G. W. Daughdrill, *Nucleic Acids Res.* **2005**, *33*, 2061–2077.
[57] D. Vuzman, M. Polonsky, Y. Levy, *Biophys. J.* **2010**, *99*, 1202–1211.
[58] J. D. Klemm, C. O. Pabo, *Genes Dev.* **1996**, *10*, 27–36.
[59] N. Khazanov, Y. Levy, *J. Mol. Biol.* **2011**, *408*, 335–355.
[60] Y. Takayama, D. Sahu, J. Iwahara, *Biochemistry* **2010**, *49*, 7998–8005.
[61] D. Vuzman, A. Azia, Y. Levy, *J. Mol. Biol.* **2010**, *396*, 674–684.
[62] L. Zandarashvili, F. Huang, A. R. Fersht, L. A. Mirny, A. M. van Oijen, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E1724–1732.
[63] J. Iwahara, Y. Levy, *Transcription* **2013**, *4*, 58–61.
[64] D. Vuzman, Y. Levy, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21004–21009.
[65] A. J. Levine, M. Oren, *Nat. Rev. Cancer* **2009**, *9*, 749–758.
[66] K. H. Vousden, C. Prives, *Cell* **2009**, *137*, 413–431.
[67] D. Vuzman, Y. Levy, *Mol. BioSyst.* **2012**, *8*, 45–57.

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