BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

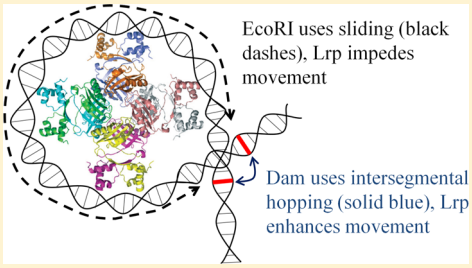
DNA Adenine Methyltransferase Facilitated Diffusion Is Enhanced by Protein−DNA "Roadblock" Complexes That Induce DNA Looping

Adam J. Pollak and Norbert O. Reich*

Department of Chemistry and Biochemistry, University of California at Santa Barbara, Santa Barbara, California 93106, United States

Supporting Information

ABSTRACT: The genomes of all cells are intimately associated with proteins, which are important for compaction, scaffolding, and gene regulation. Here we show that pre-existing protein−DNA complexes (roadblocks) diminish and——interestingly——enhance the ability of particular sequence-specific proteins to move along DNA to locate their binding sites. We challenge the bacterial DNA adenine methyltransferase (Dam, recognizes 5′-GATC-3′) with tightly bound EcoRV ENase−DNA complexes, which bend DNA. A single EcoRV roadblock does not alter processive (multiple modifications) methylation by Dam. This result disfavors a reliance on heavily touted mechanisms involving sliding or short hops for Dam. Specific conformations of two EcoRV roadblocks cause an *increase* in processivity. The histone-like leucine-responsive regulatory protein (Lrp) binds DNA nonspecifically as an octamer, and also increases Dam's processivity. These results can be explained by our prior demonstration that Dam moves over large regions (>300 bp) within a single DNA molecule using an "intersegmental hopping" mechanism. This mechanism involves the protein hopping between looped DNA segments. Both roadblock systems can cause the DNA to loop and therefore facilitate intersegmental hopping. For Lrp, this only occurs when the Dam sites are separated (by >134bp) such that they can be looped around the protein. Intersegmental hopping may well be a general mechanism for proteins that navigate long distances along compacted DNA. Unlike Dam, *Eco*RI ENase (recognizes 5′-GAATTC-3′) relies extensively on a sliding mechanism, and as expected, Lrp decreases its processivity. Our systematic use of protein roadblocks provides a powerful strategy to differentiate between site location mechanisms.



The search for specific sites by DNA binding/modifying proteins is a ubiquitous biological phenomenon.[1,2] Examples of biologically and biomedically relevant proteins that move by diffusion (without ATP) along DNA to locate their inconspicuous sites of action include DNA methyltransferases,[3,4] transcription factors,[5,6] restriction endonucleases,[7−9] and DNA repair enzymes.[10,11] In general, nonspecific DNA overwhelmingly outnumbers specific sites, and this can be as little as ∼100 fold and up to more than a million fold.[10,12,13] Various mechanisms contribute to this facilitated diffusion process, where movement along nonspecific DNA by proteins is critical for the labyrinthine site locating task, as originally reported in the 1970s.[14]

Interestingly, how proteins move along DNA given the litany of other DNA binding/bending proteins that crowd and manipulate DNA remains relatively experimentally unexplored. For example, *Escherichia coli* leucine-responsive regulatory protein (Lrp) is one of over a dozen bacterial nucleoid-associated proteins (NAPs) whose overwhelming presence coats ∼30% of the bacterial genome[15−17] and therefore certainly impacts facilitated diffusion. High cellular copy numbers and high affinities for nonspecific DNA[18] allow NAPs to simultaneously control DNA structure and gene regulatory processes; Lrp, for example, controls ∼10% of *E. coli* gene expression.[17] The coverage of genomic DNA in eukaryotes (by histones) is much more extensive.[17] One might intuit—and it has been suggested[19,20]—that these DNA-structure-manipulating-proteins will confound the site finding process; however, we interestingly demonstrate here that this is not necessarily the case, as has been invoked by others.[21−24]

In vitro processive catalysis studies are commonly used to deduce the details concerning translocation mechanisms, where the ability to carry out two modifications within a single binding event between the enzyme and the DNA is quantified.[3,9−11] How processivity changes with changes in the spacings between the sites on the DNA is critical for identifying and discriminating between specific facilitated diffusion mechanisms.[2] Sliding is one mechanism, where the protein makes a series of single base pair (bp) shifts to travel along the DNA, maintaining close contact with the DNA.[9,10] Another mechanism, hopping, involves a series of dissociation−reassociation steps, allowing the protein to move larger distances (hops) during each step.[9,10] For sliding, the drop in processivity corresponding with increases in the spacings between the two sites is sharper than for hopping.[9,10] Both sliding and hopping rely on protein movement along the trajectory of the helix (Figure 1A) and are touted as the main mechanisms responsible for facilitated diffusion.[1,11,22]

Received: October 28, 2014
Revised: March 13, 2015
Published: March 18, 2015

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

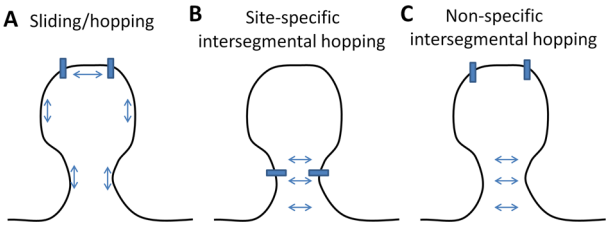


Figure 1. Facilitated diffusion mechanisms. Rectangles are sequence-specific DNA binding sites for a protein of interest. Arrows depict the trajectory of protein movements along DNA. (A) Well-studied sliding and hopping mechanisms involve movement along the trajectory of the DNA helix. (B) Site-specific intersegmental hopping takes advantage of when DNA loops involve sites that can be looped proximal in space. (C) Nonspecific intersegmental hopping involves the movement between looped segments that lack sites. For Dam, this can cause increases in processivity even when sites are closely spaced. However, this mechanism is likely helpful for proteins that need to search for sites that can be quite rare.

Several emerging lines of evidence suggest that translocation mechanisms other than sliding and hopping exist, involving large, "3-D" movements. For example, the ability of DNA to form loops, allowing distal (>300 bp) DNA segments from the same molecule to become proximal, provides proteins an alternative avenue for translocation and results in efficient long-range movements (Figure 1B,C).[3] The passive looping of DNA is dependent on its persistence length, where DNA segments less than 150 bp are rigid rods,[25] and segments 200−600 bp are increasingly able to form loops.[26,27] The protein can move between these looped DNA segments using a mechanism we refer to as intersegmental hopping,[3] which is a specific case of hopping where a hop happens between a DNA loop. This mechanism accommodates what some have called "3-D" movements or "jumps".[3] Recent evidence for this mechanism has been reported for EcoRV ENase (EcoRV),[28,29] *Eco*RI ENase (*Eco*RI),[30] alkyl adenine glycosylase (AAG),[31] and DNA adenine methyltransferase (Dam).[3] Intersegmental hopping enhances processive catalysis for Dam when the two sites are far apart enough to be looped into proximity, called "site-specific intersegmental hopping," which optimally occurs with a ∼500 bp separation between sites (Figure 1B). Intersegmental hopping also enhances processivity when the transition is between segments of nonspecific looping DNA;[3,29] this "nonspecific intersegmental hopping" causes increases in processivity for Dam even when the two sites are close (Figure 1C), involving what is probably quite a circuitous path. Increases in processivity when the distances between or surrounding sites are increased (due to intersegmental hopping) to the best of our knowledge have only been demonstrated for Dam,[3] but may well be a common strategy since many proteins are required to scan much larger regions of DNA than is typically studied *in vitro*. Intersegmental hopping has been argued to be distinct[3,29−31] from the well-studied intersegmental transfer mechanism,[5,6,32] which necessitates the formation of a ternary complex with either a monomeric protein with two DNA binding sites, or a protein complex.

E. coli Dam is an orphan methyltransferase and uses AdoMet to methylate the N^6 position of adenine in 5′-GATC-3′ sites.[33] We recently demonstrated that Dam relies heavily on intersegmental hopping during processive catalysis, while not primarily using the more typically observed sliding or hopping mechanisms.[3] However, its biological roles may help explain its translocation properties. Its participation in mismatch repair, involving the methylation of ∼20 000 GATC sites, proceeds effectively with only ∼100 Dam molecules per cell.[13,34] Dam's inability to processively methylate closely spaced sites[3] is likely inconsequential, as not all GATC sites are likely required to be methylated for mismatch repair to be effective,[35,36] and skipping sites by traveling through DNA loops is ideal to efficiently cover the breath of the bacterial genome. Furthermore, Dam's function in epigenetic gene regulation involves only ∼50 GATC sites that are clustered in certain regulatory regions[37] and are demonstrated to undergo nonprocessive catalysis. DNA binding/modifying proteins will utilize different combinations of the available translocation mechanisms, likely based on their biological context.[3]

Previous studies have utilized a "roadblock" approach to probe translocation mechanisms, where modulations of a protein's ability to travel between two sites and undergo processive catalysis are monitored with respect to the presence (and absence) of a preincubated protein roadblock.[10,36,38] For example, O'Brien and colleagues concluded alkyl adenine glycosylase (AAG) utilizes hopping as a catalytically inactive *Eco*RI roadblock only partially disturbs the processive catalysis by AAG.[10] Here we use an ensemble of highly stable complexes as well as transient complexes with two proteins (Dam and *Eco*RI) suggested to have very distinct translocation mechanisms.[3] Furthermore, we employ a histone-like Lrp roadblock in the interest of probing a more biologically relevant situation for studies of facilitated diffusion. Our systematic approach provides a powerful way to distinguish between translocation mechanisms and provides further evidence in support of the relevance of the intersegmental hopping mechanism.

MATERIALS AND METHODS

Processivity Assays. (Dam) Reactions were done in methylation reaction buffer (MRB: 100 mM Tris HCl: pH 8.0, 10 mM ethylenediaminetetraactetic acid (EDTA), 1 mM dithiothreitol (DTT), 0.2 mg/mL bovine serum albumin (BSA)) with 400 nM DNA, 5 mM $CaCl_2$ (only for EcoRV ± reactions), 7 nM Dam, and 30 μM AdoMet (*S*-adenosyl methionine, SAM) at 37°. Roadblocks (where applicable) were preincubated for 20 min prior to the initiation of the reaction by Dam. Single site EcoRV roadblock experiments (Figure 4) included 600 nM EcoRV dimers. Two site EcoRV roadblock experiments (Figure 5) included 800 nM EcoRV dimers. Lrp roadblock experiments (Figures 6 and 7) included 400 nM Lrp octamers. Dam was diluted in protein dilution buffer (20 mM potassium phosphate, 200 mM NaCl, 0.2 mM EDTA, 0.2 mg/mL BSA, 2 mM DTT, and 10% glycerol). Aliquots of the reaction mixture were heat inactivated in 75° water, which has previously been shown to be effective.[3,37] After slow cooling, NEB DpnII buffer and DpnII enzyme was added, and the mixture was incubated at 37° for 2 h. The reaction products were analyzed using PAGE (7.5% 29:1 acrylamide/bis-(acrylamide)).

(*Eco*RI ENase) Reactions were done in endonucleases reaction (ERB) buffer: 20 mM Tris: pH 7.4, 5 mM $MgCl_2$, 0.2 mM EDTA, 0.05 mg/mL BSA) with 400 nM DNA, 65 mM NaCl, and 1 nM *Eco*RI ENase at 37°. Lrp roadblocks (400 nM octamers, where applicable) were preincubated for 20 min prior to the initiation of the reaction by *Eco*RI. Reactions were quenched in 50 mM EDTA then run on a PAGE gel as above.

Gel Shifts. Lrp was added to both MRB and ERB (see above) with 400 nM DNA for 20 min. The mixture was

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

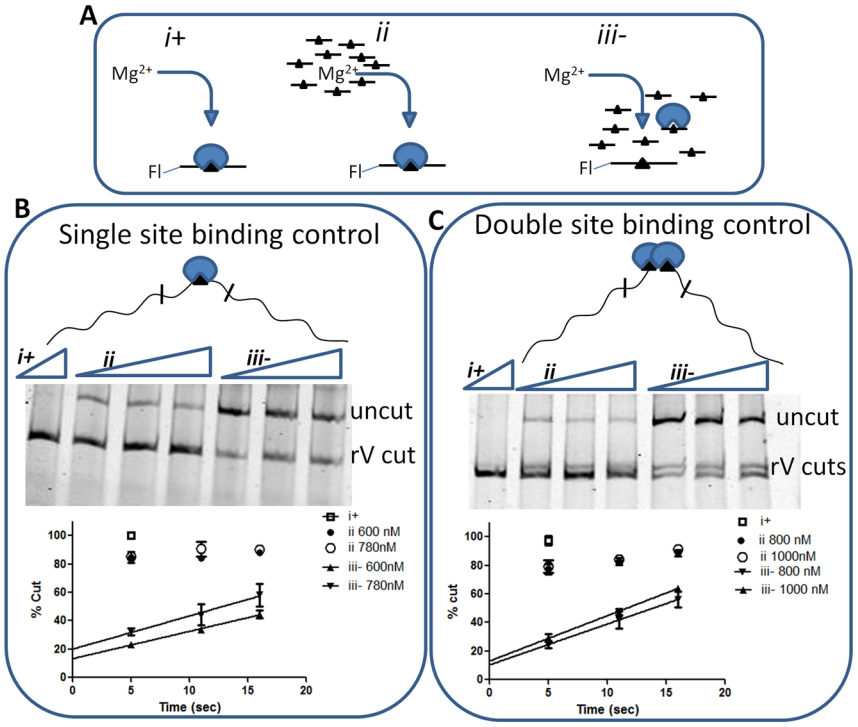


Figure 2. Confirmation of site-specific occupancy of EcoRV ENase. (A) Schematic of three experiments (see text for details): (i) A preincubated EcoRV, Ca^{2+}, and DNA (fluorescein labeled (-Fl)) complex undergoes cutting with the addition of Mg^{2+}. (ii) The cutting proceeds in spite of the addition of excess DNA chase (unlabeled) which is added simultaneously with the Mg^{2+}, suggesting that EcoRV remains bound at its site. Increasing enzyme shows no added cutting, displaying enzyme saturation. (iii) The lack of a burst of product formation from EcoRV preincubated with chase DNA shows that the chase is capable of partitioning the enzyme, if it had left the site in ii. (B) Demonstration of burst data for i–iii by electrophoresis for single site (276 mer), and (C) for double site substrate (276 mer, 17 bp between EcoRV sites). The chase DNA (35 bp) is 5 μM.

quenched with an equal volume of 50% glycerol and immediately run on a gel.

EcoRV Binding Chase Assay. 400 nM DNA (rV42 in Figure 2B, 2rV17 in Figure 2C) was added into MRB with 5 mM $CaCl_2$ at 4°. EcoRV was added at indicated concentrations. $MgCl_2$ was added to initiate reactions to a total concentration of 10 mM. In ii, $MgCl_2$ was mixed with unlabeled chase DNA, which was simultaneously added generating a total of concentration of 5 μM chase DNA. In iii, the chase DNA (total concentration of 5 μM) was added prior to the addition of the EcoRV.

Enzyme Expression and Purification. Dam was purified exactly as before.[37] EcoRV was kindly provided by Dr. John Perona and was purified as described.[39] Lrp was purified as previously described.[18] *Eco*RI ENase was purchased from NEB.

DNA Substrates. See Supporting Information.

Data Analysis. The processivity values were derived from a least-squares fitting using Microsoft excel. Gels were scanned on a Typhoon phosphoimager (GE). *P* values: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

RESULTS

Demonstration of Site-Specific EcoRV Occupancy As a Roadblock. We employ EcoRV as a roadblock. EcoRV is a type II restriction endonuclease that recognizes and cuts both strands of 5′-GATATC-3′ as a dimer upon addition of the Mg^{2+} cofactor.[39,40] EcoRV binds its sites several orders of magnitude more tightly than nonspecific DNA in the presence of Ca^{2+},[39,41] which forms a stable noncatalytic complex with a very slow off rate,[41] allowing it to maintain its position throughout the time course of our reaction. We are also interested in EcoRV's ability to bend DNA ~50°,[39] as this expands the scope of possible DNA configurations to explore with processivity experiments. This bending estimate is consistent with both crystallographic and solution studies.[40,41] The integrity of roadblock experiments critically relies on evidence of site-specific occupancy.

We adopt a catalytic DNA cutting chase assay to confirm site-specific binding by EcoRV.[10] EcoRV displays burst kinetics, where the cleavage of DNA by a preincubated enzyme–DNA complex is faster than the subsequent catalytic cycle, where product release is the rate limiting step.[42] The chase DNA is unlabeled, and the DNA which is preincubated with EcoRV is labeled. The ability to cut the labeled DNA in the presence of chase is indicative of its specific binding as described in the following experiments (i–iii) (Figure 2A). In (i) enzyme is in slight excess and in the presence of Ca^{2+} is incubated with DNA, and cleavage is initiated with excess Mg^{2+}, resulting in total cleavage of the DNA (Figure 2B). The enzyme appears to exchange the divalent metal cofactors without perturbations of the DNA–protein complex, consistent with prior reports.[41] In (ii) Mg^{2+} and excess chase DNA are simultaneously added to initiate the cutting reaction. This experiment addresses the possibility that EcoRV leaves the site but can rapidly return (which turns out not to be the case). Importantly, nearly all of the DNA is cut, showing the enzyme remains bound to the DNA (Figure 2A). The addition of more EcoRV does not result in further cutting, demonstrating EcoRV saturation (Figure 2B). In (iii) the chase and the labeled DNA are preincubated with EcoRV prior to the initiation of Mg^{2+}. The minimal burst of cut labeled DNA is indicative of the effectiveness of the excess chase DNA, where the cutting of

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181–2192

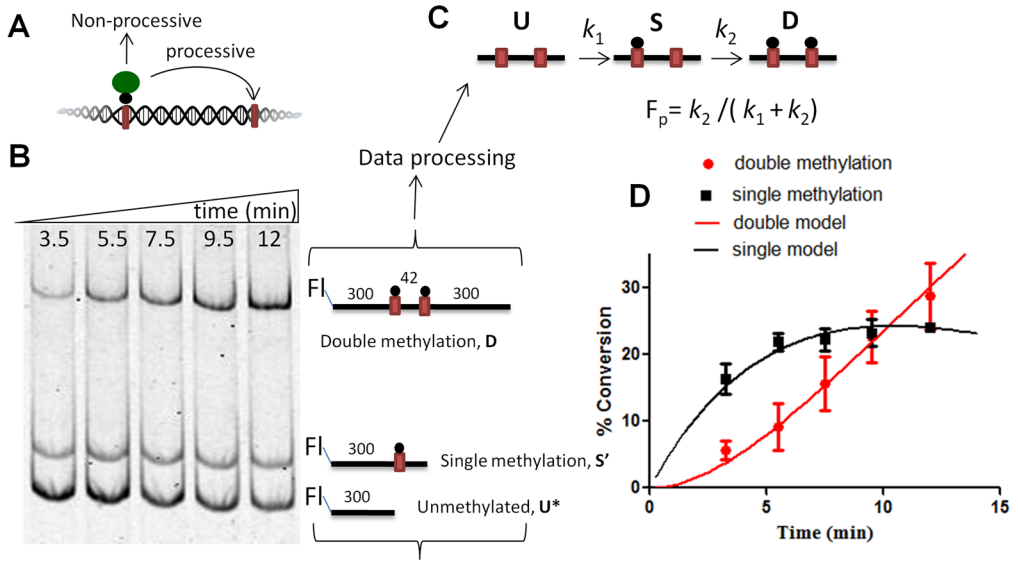


Figure 3. Processivity assay: (A) Dam (green circle) immediately following the first methylation (black circle) at a GATC site (bold red lines). Processivity is based on the probability of a second methylation event following an initial one during a single binding event. (B) The reaction includes 7 nM Dam, 400 nM DNA (symmetric substrate, fluorescein labeled (-Fl)), 30 μM AdoMet (*S*-adenosyl methionine), 30 mM NaCl, 5 mM $CaCl_2$, and buffer at 37°. Following the methylation reaction, heat quenched samples were digested with DpnII endonuclease (cuts unmethylated GATC sites), and reaction products were separated by nondenaturing PAGE and imaged using a typhoon phosphoimager. Upon manipulations,[3] the densitometry provides the relative amounts of un-, singly, and doubly methylated DNA at each time point. (C) The relative amount of each species of the reaction (S and D) is fit to a sequential reaction mechanism model (eqs 1−3) to derive the rate constants k_1 and k_2, and ultimately the processivity value (F_p). (D) Fit of data to model for substrate with 42 base pairs between sites. Mean and SD of ≥4 independent experiments are shown.

the labeled DNA proceeds through the slow steady-state cycle (Figure 2B). These experiments (i−iii) were repeated with a two site substrate, again providing evidence of site specific occupancy (Figure 2C). Additional evidence for site-specific binding is described below. Interestingly, the decrease in (ii) with respect to (i) is likely due to the delay in the cutting of the second strand (within one site) which may allow the capture of the enzyme by the chase DNA, as has been seen previously.[10]

Processivity Assay. Briefly (our previous publication describes this assay in detail[3]), the processivity assay involves excess DNA (400 nM) with two Dam recognition sites, and low enzyme (7 nM) to drive conditions where one enzyme or less binds to a single DNA molecule (Figure 3).[3] The reaction is initiated with the enzyme under multiple turnover conditions, and aliquoted time points are heat quenched, slow cooled, and then subjected to a DpnII digestion, which cleaves unmethylated Dam sites (which has been shown to be complete[3]). PAGE analysis allows the determination of the relative amounts of unmethylated, singly methylated (S), and doubly methylated (D) DNA at each time point (Figure 3B,C). A minimal accumulation of singly methylated products corresponds to a highly processive reaction, and a large accumulation of singly methylated products corresponds to a nonprocessive reaction.

The processivity data is quantified using a sequential methylation scheme, where k_1 is the first order rate constant from unmethylated to singly methylated DNA, and k_2 is the first order rate constant from singly to doubly methylated DNA (eqs 1 and 2) (Figure 3C).[43]

$$\text{singly methylated (S)} = \frac{k_1}{(k_2 - k_1)}(e^{-tk_1} - e^{-tk_2}) \quad (1)$$

$$\text{doubly methylated (D)} = 1 + \frac{1}{(k_1 - k_2)}(k_2 e^{-tk_1} - k_1 e^{-tk_2}) \quad (2)$$

Processivity (F_p) (the relevant outcome of the analysis) relates the values of k_1 and k_2 (eq 3), which are ultimately derived from the gel densitometry as discussed above and previously.[3]

$$F_p = \frac{k_2}{k_1 + k_2} \quad (3)$$

When $k_2 \gg k_1$ processivity is 1.0, which is maximal. When $k_2 = k_1/2$, processivity is 1/3, which is minimal.[3]

Dam's Processivity Is Unaffected by an EcoRV Roadblock. EcoRV binds DNA as a dimer, and the complex has a ∼5 nm diameter.[40,44] It almost completely encloses the DNA and therefore could sterically block an enzyme sliding along the DNA (Figure 4A),[40] as has been demonstrated previously with a similar noncatalytic *Eco*RI roadblock.[36] Dam's processivity is minimally reduced by the EcoRV roadblock, as EcoRV's absence and presence results in small modulations in processivity for a substrate with 42 bp separating the two Dam sites (rV42), with the EcoRV site positioned in the middle of the two Dam sites (Figure 4B,C, Table 1). The overall rate of the reaction is similar with and without roadblock (see below). Processivity is also minimally reduced using a substrate with 150 bp between the Dam sites and the EcoRV site in the middle (Figure 4D). This longer substrate (rV150) has a higher base-level (no roadblock) processivity, due to its greater DNA loop formation propensity.[3]

These processivity results suggest that Dam does not utilize sliding or short hops (which are likely to be disturbed by such a roadblock[10]) and are provocative considering prior reports, where similar roadblocks vary from partially to completely

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

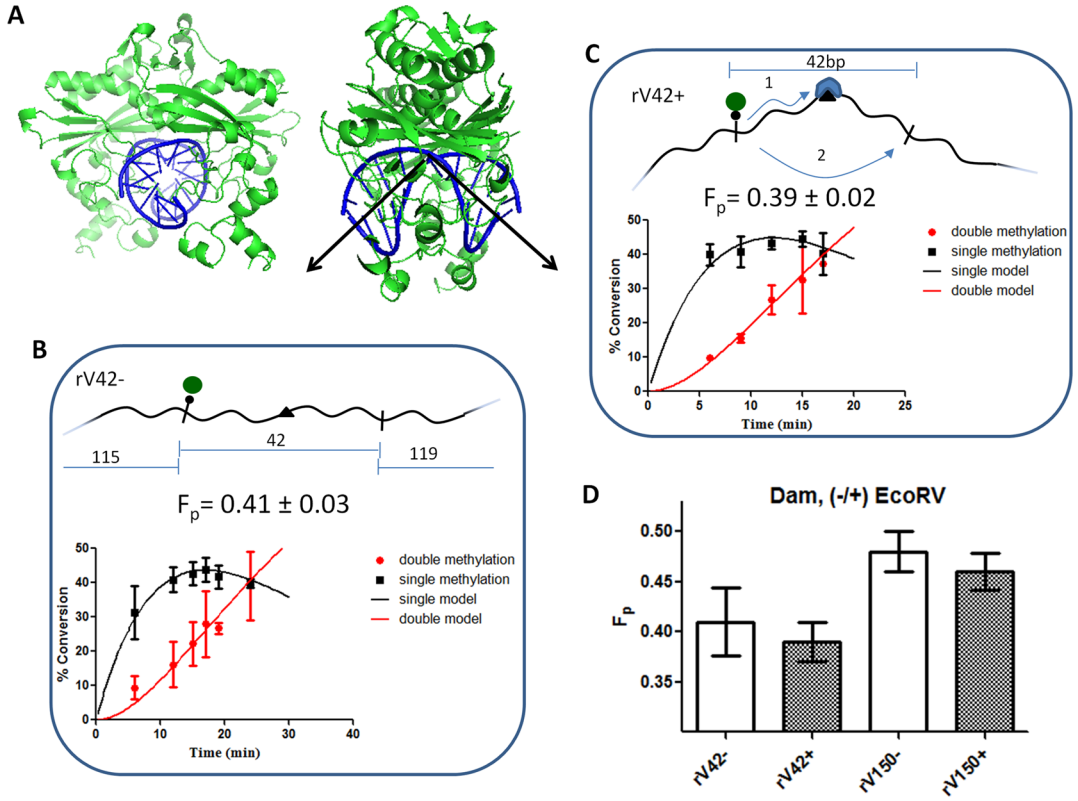


Figure 4. Dam is processive in spite of an EcoRV roadblock. (A) Structure of EcoRV dimer bound to cognate DNA (PDB 2RVE)[40] (left). Note the majority of the DNA is enclosed by this "saddle" dimer. EcoRV bends DNA ~50°, sharply between the T-A step (right). Arrows show the trajectory of the DNA from the bending locus. (B) Dam's (green circle) processivity in the absence (−) of the roadblock (the site is a black triangle) (rV42-: 42 bp between Dam sites, single EcoRV roadblock site, 276 mer total size). (C) Processivity in the presence (+) of the EcoRV roadblock (blue shape) is relatively unchanged in comparison to B. The enzyme therefore likely does not travel along the helix using sliding or hopping (path 1). (D) F_p data from B and C is plotted. The lack of effect of the roadblock is similar when the distance between the Dam sites is larger (rV150 ±: 150 bp between Dam sites, single EcoRV roadblock site, 384 mer total size). This suggests that Dam is unlikely to utilize sliding and hopping mechanisms, and instead favors an intersegmental hopping mechanism. Mean and SD of ≥4 independent experiments are shown. *P* values (± roadblock) are n.s.

Table 1. Names and Schematics of DNA Substrates[a]

Name	Substrate	Name	Substrate
rV42		Dam42Lrp	
rV150	150	Dam42LrpL	300 300
2rV12	12	Dam134Lrp	134
2rV17	17	Dam284Lrp	284
2rV21	21	Eco42Lrp	42
2rV12L	300 12 300	Eco134Lrp	134
2rV17L	300 17 300		
2rV21L	300 21 300		

[a]Dam sites: upright black lines; EcoRV sites: black triangles; EcoRI sites: upright blue lines. A 42 bp distance is between the Dam sites and 115/119 bp of DNA are flanking those sites unless indicated otherwise. Note: The substrates are not drawn to scale.

inhibiting movement between sites.[10,36] However, our data are consistent with previous results for Dam, which suggests its limited ability to track along the trajectory of the helix by sliding or hopping.[3] A probable interpretation of this result invokes intersegmental hopping by DNA looping (see introduction), which we predict would not be significantly disturbed by a roadblock. This involves the enzyme making a series of nonspecific intersegmental hops involving DNA

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

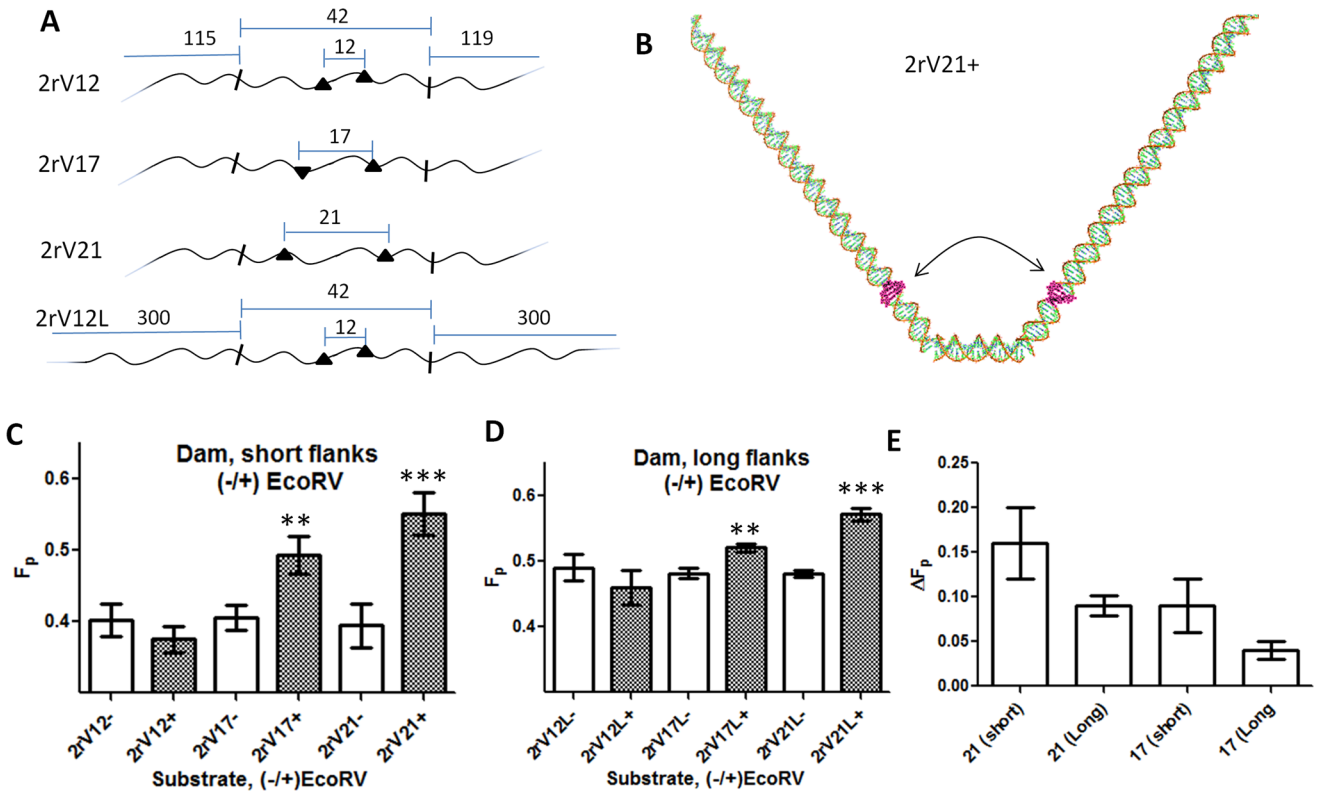


Figure 5. Dam processivity increases as a consequence of an EcoRV mediated enhancement in DNA looping. (A) Schematic of the substrate series where the distance between Dam sites remains constant, yet the EcoRV roadblock sites (black triangles) are modulated. (B) Schematic of 2rV21+ DNA using the 3-D Dart program.[47,48] The EcoRV induced bending of the DNA likely enhances loop formation and subsequently Dam's ability to undergo intersegmental hopping. (C) Processivity for Dam with and without EcoRV for the substrates in A (short flanks, the first 3), which here are 276 bp long. Processivity is most increased when DNA looping is optimal 2rV21+. (D) Long DNA flanks (642 bp total substrate size) enhance Dam's ability for intersegmental hopping in the absence of roadblocks. EcoRV roadblocks offer more modest increases in processivity due to the higher baseline processivity levels. (E) ΔF_p from 5C (short flanks) and 5D (long flanks). Since ΔF_p decreases, the source of processivity enhancement is likely due to looping, and not by some other mechanism. Mean and SD of ≥4 independent experiments are shown. P values (± roadblock) are displayed or are n.s. *P* values unreported for E.

looping, between DNA regions that mostly lack sites (Figure 1C), transitioning primarily on the DNA flanks, eventually resulting in processive catalysis.

The EcoRV roadblock may have pleiotropic effects, where the protein-induced bending of the DNA may enhance looping (and therefore processivity), but the roadblock may in other ways diminish processivity, for example, by interacting with the traveling Dam enzyme. Our data seem consistent with an intersegmental hopping mechanism; however, experiments further modulating the ability of DNA to form loops (and enhance processivity) were carried out to provide a clearer mechanistic interpretation. Furthermore, minimal effects on Dam reaction rates by EcoRV suggest limited interactions between Dam and EcoRV (see below).

Two EcoRV Roadblocks *Increase* Processivity by DNA Looping. The lack of an effect on Dam's processivity with a single EcoRV roadblock motivated us to probe the potential processivity modulations from various configurations of two EcoRV roadblocks. We hypothesized two potential outcomes: (1) the processivity will decrease due to the roadblock(s) impeding hopping, as has been shown for other proteins.[10] Dam may have been able to hop over one centrally located roadblock (Figure 4D), but the sterics of two roadblocks positioned various distances from the Dam sites may block the enzyme and diminish processivity. (2) The EcoRV induced bending of DNA is additive and will facilitate DNA looping; this may enhance intersegmental hopping and therefore increase processivity. The substrates with the two EcoRV sites are shown in Figure 5A (Table 1), where the Dam sites are separated by 42 bp for each.

The phasing of DNA is 10.5 bp, and bends that are separated by such phasing have been shown to be additive and therefore can dramatically increase DNA loop formation in a variety of systems.[45,46] Substrate 2rV21+ includes this optimal phasing. Using the 3D-DART program (part of the HADDOCK system),[47,48] we generated a model of 2rV21+ by manually introducing the 50° bends due to the EcoRV into the DNA structure (Figure 5B). The phasing of the substrate places the DNA flanking the EcoRV bends in the same general space, allowing the GATC sites to be positioned close in space such that a combination of nonspecific intersegmental hopping and possibly site-specific intersegmental hopping can occur.

As expected, the processivity values for 2rV12-, 2rV17-, and 2rV21- are similar (Figure 5A,C). Consistent with hypothesis 2, Dam's processivity increases in the presence of the roadblocks. We also note that the differing processivity outputs based on modulations of where the EcoRV sites are further evidence of site-specific binding of the EcoRV roadblock(s); however, our mechanistic interpretation stands even if a small portion of the enzyme travels away from the site. The most significant increase in processivity is seen with 2rV21+ which is consistent with the superstructured substrate in Figure 5B. The

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

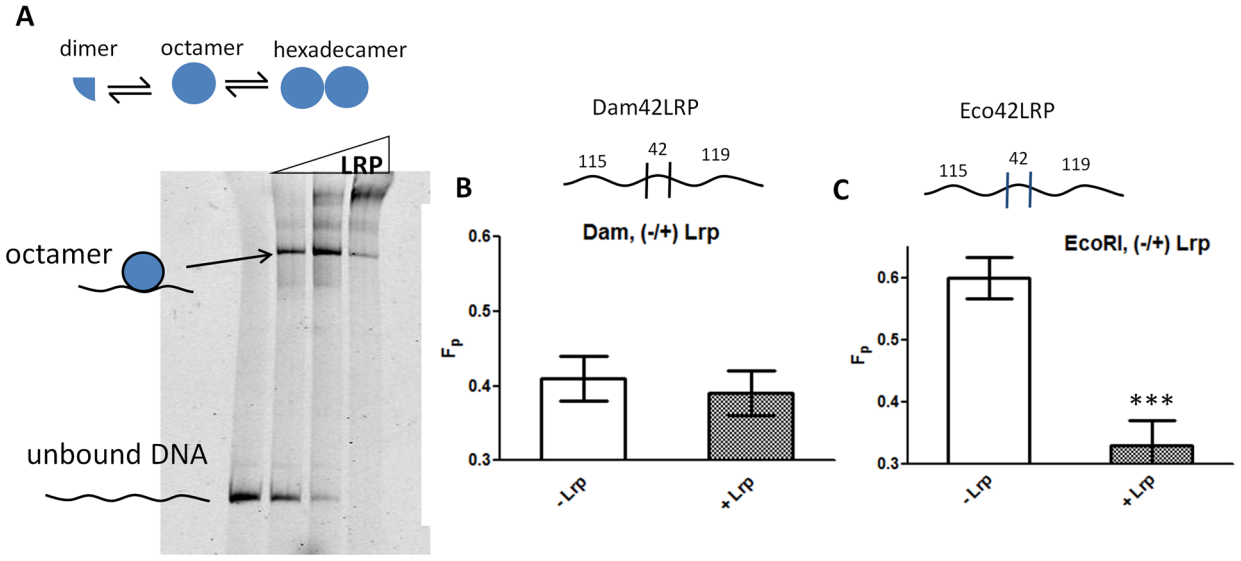


Figure 6. Histone-like Lrp roadblock reveals a translocation mechanism dependent processivity response (A) Lrp is capable of dynamic oligomerization and can bind tightly to nonspecific DNA as an octamer. Gel shows stable nonspecific DNA-octamer complex. From left to right the lanes include 0, 0.5, 1, and 2 Lrp octamers per DNA. (B) Dam's processivity is minimally affected by the Lrp roadblock. (C) *Eco*RI ENase significantly utilizes sliding and is nearly maximally processive on a substrate with 42 bp site separations.[3] In the presence of Lrp, *Eco*RI's ability to undergo processive catalysis is essentially completely abolished. This demonstrates that Lrp can disrupt processivity. Mean and SD of ≥4 independent experiments are shown. *P* values (± roadblock) are displayed or are n.s.

processivity data show a clear trend with processivity increasing as the roadblocks approach the Dam sites. By orienting the Dam sites and the flanking DNA toward the center of the substrate, the ability of the protein to exchange between DNA flanks and Dam sites should be enhanced and cause an increase in processivity. As a control, we generated a roadblock system nearly identical to the one in Figure 5, yet involving no induced DNA bends (Figure S1, Supporting Information). Importantly, when the roadblocks are separated by 21 bp, processivity is slightly reduced, consistent with the notion that the bending of the DNA is critical for processivity enhancements (Figure S1). To further show that the enhancement in processivity observed with this set of EcoRV substrates (Figure 5) is not due to the proximity of the EcoRV protein to the Dam site, we designed the following set of experiments.

Modulating DNA Flanks Provides Evidence for Intersegmental Hopping by EcoRV Roadblocks. We sought to challenge the hypothesis that the enhancement in processivity in Figure 5 is due to DNA bending-mediated intersegmental hopping, and not by some other mechanism, involving, for example, an interaction between the EcoRV roadblock and Dam, or allosteric modulation of the Dam site through the DNA upon EcoRV binding.[49] We consider this in part because of the proximity of the EcoRV sites to the Dam sites. We chose to lengthen the DNA surrounding the Dam sites on the substrates from the previous section (Figure 5A) that contain the two EcoRV roadblock sites separated by various amounts (Figure 5A). The lengthening of the flanking DNA increases processivity in the absence of roadblocks (Figure 5D) as the longer DNA flanks enhance DNA looping (as has been previously demonstrated[3]). We are interested in how the ΔF_p values (the F_p in the presence minus the F_p in the absence of roadblock) change between the current (Figure 5D, longer) and prior (Figure 5C, shorter) substrate set. We propose two possible outcomes: (1) If intersegmental hopping is involved, the ΔF_p with the longer substrates will be less. This is because the longer flanks enhance processivity, and the bending of the flanks toward each other may be redundant. (2) If another mechanism contributes to roadblock mediated enhancing of processivity, the ΔF_p will be the same regardless of the changes in flanking DNA.

Our data support hypothesis 1, where the ΔFp is less for the substrates with the longer flanks (Figure 5E). The inclusion of the roadblocks, with or without the flanks appears to maximize the enhancement in processivity as mediated by intersegmental hopping. For example, lengthening the flanks in Figure 5B is inconsequential based on similar F_p values for the 2rV21+ and 2rV21L+ (Figure 5C,D).

Nonspecific Lrp Binding Disturbs Processivity for *Eco*RI, but not Dam. How do transient protein complexes differentially impact translocating proteins which rely on sliding versus intersegmental hopping? Here we rely on Lrp, a well-studied NAP. The Lrp−DNA complex moves our investigation toward a more biologically relevant situation. We confirmed conditions where Lrp, most likely as an octamer, binds DNA nonspecifically by electrophoresis (Figure 6A).[18] Similar tight banding (lack of smearing) of both nonspecific and specific DNA octamer complexes for Lrp has been demonstrated.[18] The lack of a smeared octamer band (Figure 6A) suggests that the Lrp is likely bound near the center of the DNA, as was demonstrated with the specific complex.[18] If Lrp binds near the ends and in the center of the DNA, the banding would be much more smeared as different complexes have distinct gel mobilities.

Previous work has demonstrated that Dam's catalysis is relatively unaffected by nonspecifically bound Lrp.[50] Even though catalysis also involves the searching of the DNA for the Dam site, we sought to extend this previous result using our processivity analysis here. Dam's processivity is largely unaffected by the nonspecifically bound Lrp roadblock for a substrate with 42 bp between the sites (Figure 6B, Table 1). However, the transient nature of the Lrp−DNA nonspecific complex invokes the possibility that it is unable to block any type of facilitated diffusion process, which we address next.

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

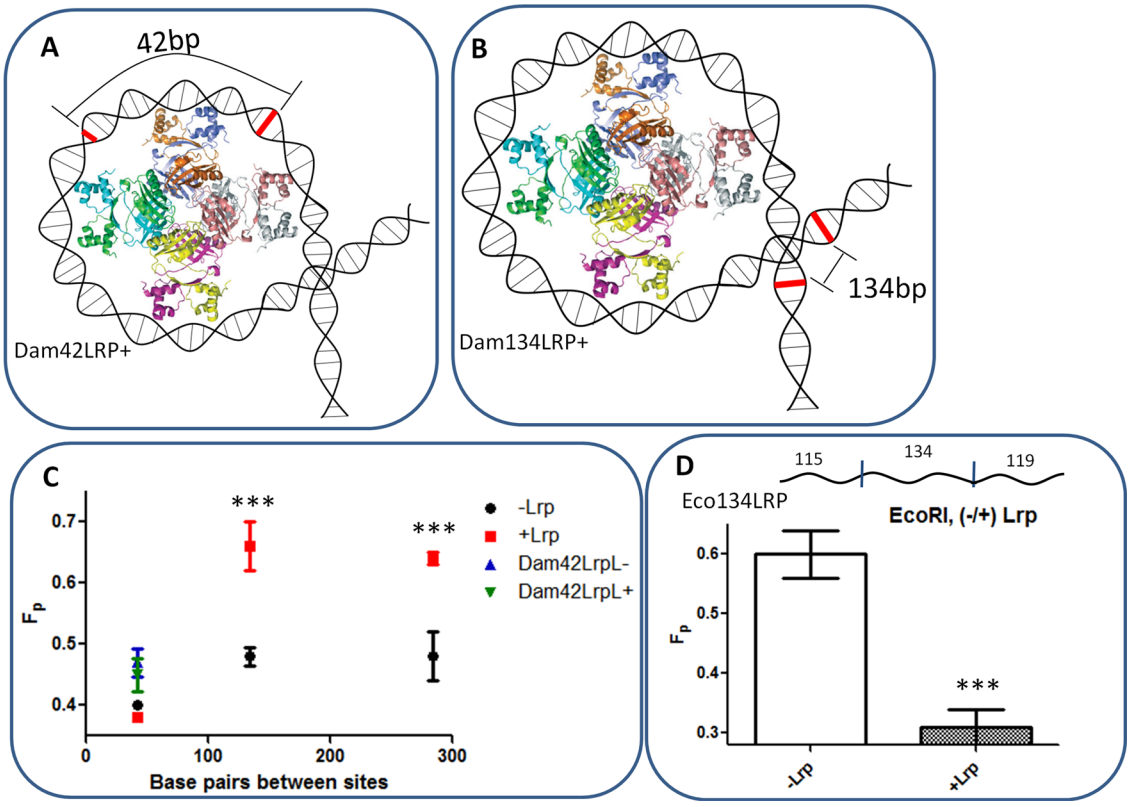


Figure 7. Lrp enhances Dam processivity only when it is able to loop Dam sites into proximity. (A) The crystal structure of *E. coli* Lrp (2GQQ) with DNA modeled in. ~130 bp of DNA are wrapped around Lrp.[52] The Dam sites (red) are ~42 bp apart. (B) The same Lrp complex (from A) with DNA where the distance between Dam sites is ~134 bp. When the sites are far enough separated on DNA, it becomes possible that Lrp loops the Dam sites proximally. (C) When the Dam sites are separated by 134 bp and 284 bp, Lrp's looping increases Dam's processivity. For two substrates when the Dam sites are 42 bp apart processivity is not enhanced by Lrp: (1) replot of data from Figure 6B (red/black, no error); (2) the substrate called "flanks" (blue/green), which refers to a substrate with 300 bp flanks on either side of the Dam sites. (D) *Eco*RI's processivity on a substrate with 134 bp separating the sites is significantly decreased in the presence of Lrp, consistent with *Eco*RI's inability to utilize intersegmental hopping. Mean and SD of ≥4 independent experiments are shown. *P* values (± roadblock) are displayed or are n.s.

In part as a control to confirm the binding of the Lrp and its potential as an effective roadblock, we carried out a similar experiment probing the *Eco*RI's processivity response to nonspecifically bound Lrp. Importantly, *Eco*RI's translocation mechanism is quite distinct from Dam's.[3] Several lines of evidence suggest that *Eco*RI primarily uses a sliding mechanism,[3,8,51] where translocation proceeds with close association between the protein and the DNA.[9] Therefore, we predict that *Eco*RI's processivity will be decreased by Lrp. We previously demonstrated that due to the cutting nature of endonucleases' catalytic activity, that their processivity range is 0.33−0.58.[3,51] In the absence of the Lrp roadblock, *Eco*RI is nearly completely processive on a substrate with 42 bp site spacings (Figure 6C). Following the confirmation of the Lrp−DNA complex formation under the *Eco*RI reaction conditions by electrophoresis (similarly to Figure 6A, data not shown), we show that *Eco*RI's processivity is indeed dramatically decreased upon the addition of Lrp (Figure 6C). The processivity drop essentially spans the entire processivity range. This result confirms both the integrity of the nonspecifically bound Lrp roadblock (demonstrating that it can disrupt protein movement) and that *Eco*RI's sliding shows the expected response to the large Lrp roadblock. This provides a proof-of-principle that proteins will respond differently to roadblocks based on their translocation mechanism.

Larger Dam Intersite Separations Support Lrp Based Enhancements in Processivity for Dam. A direct challenge of Lrp's ability to enhance intersegmental hopping by DNA looping relies on experiments using different spacing between the Dam sites. The crystal structure of *E. coli* Lrp is available (the DNA cannot be resolved in the structure). DNA has been modeled around it, resulting in a ~130 bp DNA loop surrounding the octamer.[52] The hypothesized bending and looping of DNA by *E. coli* Lrp is consistent with the behavior of other proteins similar to Lrp (refs 16, 53, and 54 Discussion). Importantly, Lrp must dramatically manipulate the DNA, as ~130 bp DNA loops are nearly impossible to form in Lrp's absence. Therefore, the two Dam sites separated by 42 bp are unlikely to be accessible to Dam and looped toward one another (Figure 7A, Table 1). In contrast, when the sites are separated by 134 bp it becomes possible for the Dam sites to be looped proximally as a consequence of having the DNA looped around the Lrp octamer (Figure 7B). When separated by 284 bp the Dam sites again are unlikely to be imbedded within the Lrp−DNA complex and instead are likely to be positioned proximally along the DNA flanks.

When either 134 or 284 bp separate the sites, the processivity is increased with the presence of Lrp (Figure 7C). We stress the novelty of this result, as to the best of our knowledge this is the first demonstration that histone-like proteins can *enhance* facilitated diffusion processes. We believe this is due to the

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

ability of the Lrp to loop the segments containing the Dam sites into proximity (which is unavailable in the 42 bp Dam site separation in Figure 7A,C). As a control we tested the effect on processivity for the 42 bp separation where the flanking DNA segments are increased. Consistent with the previous result (Figure 6B), the minimal decrease in processivity due to the inability of the Dam site containing DNA segments to loop is further suggestive that the spacings between the sites are the critical parameter, not simply the length of the DNA (Figure 7C).

Finally, *Eco*RI's processivity with Lrp when the *Eco*RI sites are separated by 134 bp was explored. The similar baseline (no roadblock) processivity between 42 and 134 bp spacings is consistent with prior results demonstrating *Eco*RI's extensive use of sliding[51] (Figure 7D). The significant drop in processivity to essentially the lower limit of processivity is further suggestive of the inability of *Eco*RI to utilize intersegmental hopping (Figure 7D) and its extensive use of sliding. This result further legitimizes the interpretation that Dam's site spacing specific Lrp dependence is due to intersegmental hopping.

Roadblock Mediated Velocity Modulations. Our interest in processivity is critical to deduce translocation properties. However, the relative velocities in the presence (and absence) of roadblocks may provide information about our experimental systems. A single EcoRV roadblock (rV42 and rV150) causes a slight increase in velocity (Figure 8, Figure S2 (from Figure 4)). In general, two EcoRV roadblocks seem to have little effect on the reaction rates, and interestingly the velocity is slightly increased when the EcoRV sites are separated by 17 bp (Figure 8, Figure S2 (from Figure 5)). These results are consistent with Dam minimally interacting with EcoRV, which is likely binding tightly on its recognition site (representing a small fraction of the total DNA size). Importantly, if the EcoRV roadblocks were interfering with Dam when in close proximity (2rV21 and 2rV21L: Figure 5), the velocity would likely decrease, which is not the case; for example, the minimal velocity differential is similar between 2rV12 and 2rV21, where in the former the EcoRV sites are much farther from the Dam sites than in the latter. In contrast, Lrp lowers the rates ~2-fold for both Dam and *Eco*RI reactions (Figure 8, Figure S2 (from Figures 6 and 7)). The decrease in velocity is predictably due to Lrp's large size and extensive interactions with the DNA.

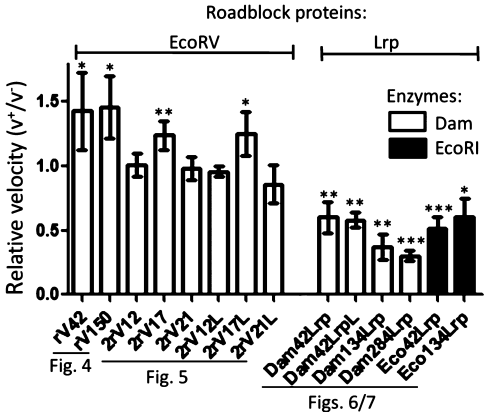


Figure 8. Reaction rates are predictably modulated by roadblock type. Velocity ($k_1 + k_2$) with roadblock is divided by velocity in the absence of roadblock from processivity experiments (Figures 4–7, Figure S1 for full velocity data). EcoRV roadblock(s) data (Figures 4 and 5) are on the left; Lrp roadblock data (Figures 6 and 7) are on the right. Dam processivity is clear bars; *Eco*RI processivity is filled bars. EcoRV does not decrease velocity while Lrp does, consistent with Lrp's significant alteration of DNA structure. *P* values (± roadblock) are displayed or are n.s. in relation to 1, i.e., no velocity modulation from the roadblock.

DISCUSSION

The majority of facilitated diffusion studies are by necessity constrained to conditions significantly removed from any biological context. Considering how the movement of proteins along DNA is impacted by other proteins is an important step toward reconciling the *in vivo* legitimacy of the detailed mechanistic insights gained from facilitated diffusion studies. Furthermore, well established sliding and hopping mechanisms have been studied with DNA molecules that are typically less than 100–200 bp, while site finding in the cell likely involves longer movements. Also, only a minority of DNA binding proteins' facilitated diffusion properties have been studied. Intersegmental hopping represents an intuitive example of previously ambiguously described 3-D movements that account for protein translocation on the order of thousands of bp. However, while growing evidence suggests that other proteins (besides Dam) likely use intersegmental hopping,[28−31] the consideration of this mechanism in the presence of roadblocks remains experimentally unexplored. In general, the extent of large, histone-like proteins, whose abundance in both bacteria and eukaryotes is massive, is only now being investigated in its relation to facilitated diffusion.[22]

HU (heat-unstable nucleoid protein), considered a prokaryotic histone homologue, is one of the bacterial NAPs that covers the *E. coli* genome.[15] HU's ~ 50 000 molecules in the cell support a separation of, on average, ~350 bp between HU oligomers.[15] HU induces DNA loop formation, where ~100-mers cyclize in the presence of nonspecifically bound HU. This involves numerous contacts between the protein and the DNA that significantly alter the structure of DNA to achieve such looping, similarly to what has been seen with *E. coli* Lrp (and related proteins).[16,52−54] These multiple structural and biochemical examples provide the basis for the schematics in Figure 7A,B. Interestingly, the 11.5 nm diameter of the related MtbLrp is similar to the 11 nm nucleosome diameter,[53] suggesting that experiments with Lrp can be extrapolated to understanding movement in the eukaryotic cell.

Our results with Dam and Lrp show that Lrp's ability to loop DNA provides an enhancement in processivity (Figure 7). Importantly, the increase only occurs when the Dam sites are sufficiently separated to be looped by Lrp, consistent with an intersegmental hopping mechanism (Figure 7). Furthermore, the two site EcoRV (Figure 5) and Lrp results (Figures 6 and 7) both demonstrate a context-specific roadblock-enhancement of Dam's processive catalysis, which taken together provides added legitimacy for these provocative results. Intersegmental hopping is an elegant way for proteins to navigate along DNA in the presence of roadblocks that may appear to confound the site finding process. Statistically, Dam sites are separated by ~250 bp in the *E. coli* genome, further suggesting the biological relevance of intersegmental hopping being enhanced by NAPs (Figure 7). In addition, known and putative Dam sites likely to undergo epigenetic gene regulation are typically clustered (<50 bp separation)[37] and are probably *unprocessively* methylated.[55] Lrp's inability to enhance the processivity of closely spaced Dam sites (Figures 6 and 7) is consistent with this.

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

Many theoretical studies have considered proteins' compaction of DNA as a mode of enhancing site finding. For example, Li and co-workers suggested that the amount of DNA binding proteins in an *E. coli* cell requires that proteins utilize a looping-based translocation mechanism.[21] These predictions are consistent with our experimental data and the notion that "roadblocks" can help with facilitated diffusion processes.[23,24] Furthermore, these studies suggest that sliding or hopping processes that involve movement along the trajectory of the helix (Figure 1A) are unlikely to contribute to site finding *in vivo*, and that many DNA binding proteins will likely use looping-based translocations that intersegmental hopping accommodates. Our results speak directly to the uncertainty in the literature regarding the potential consequences of DNA-associated proteins on facilitated diffusion properties (introduction).

As a control to our Dam-Lrp studies, we show *Eco*RI cannot bypass the Lrp roadblock (with either 42 or 134 bp separations) and consequently does not utilize intersegmental hopping as a significant contribution to its site finding process, consistent with prior reports.[3,8,51] Our results show that these Lrp experiments are able to dramatically differentiate between translocation mechanisms. Dam and *Eco*RI rely on distinctive blends of translocation mechanisms which is further demonstrated here, showing the value of these roadblocks as a tool for probing the details of translocation mechanisms for a variety of other proteins of interest.

Several techniques and approaches have been used to elucidate the details concerning translocation mechanisms, including single molecule studies.[56] The most commonly used strategy, however, involves predictable modulations in processivity values with respect to changes in the intersite spacings between target sites (introduction). Yet many enzymes have been shown to utilize a combination of sliding and hopping, and segregating the extent of each can be quantitatively and mechanistically unsatisfying (considering intersegmental hopping further complicates this). Clever modifications to such processivity assays can differentiate between translocation mechanisms.[10,31,57,58] The systematic variation of site to site distances, combined with a blocking protein (Figure 4) may be useful for studying other enzymes. For example, an enzyme's (one that uses a combination of sliding and hopping[9−11]) processivity may be decreased when the roadblock is included between closely spaced sites but will have less of an effect when the sites are farther apart. Such a trend can be informative to estimate the relative bp range for a hop or the extent of sliding, which will be relevant to the majority of proteins.

Dam's overwhelming use of intersegmental hopping is reconciled by its low copy number, need to transverse large regions of the genome,[3] and minimal need to make short movements along DNA (introduction). Dissimilarly, *Eco*RI is involved in a restriction modification system, where it cleaves invading foreign nucleic acids, such as phage DNA. Incoming phage DNA is less likely than genomic DNA to be associated with NAP's, in part to ensure the transcription of phage genes.[59,60] This allows *Eco*RI to slide to find its sites on phage DNA. *Eco*RI's inability to maneuver among histone-like roadblocks may also contribute to the prevention of cutting genomic DNA. However, other proteins (than Dam), such as many transcription factors, with low copy numbers[61] and rare binding sites,[12] may also utilize intersegmental hopping. These roadblock types of experiments may help to reveal this.

■ ASSOCIATED CONTENT

Supporting Information

Additional data as well as details regarding the construction of the DNA substrates used in this study and the rate constants seen in Figure 8. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: reich@chem.ucsb.edu; phone: (805) 893-8368.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ENase, restriction endonuclease; *Eco*RI, restriction endonulease *Eco*RI; EcoRV, restriction endonuclease EcoRV; bp, base pair; Dam, DNA adenine methyltransferase; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; NAPs, nucleoid-associated proteins; Lrp, leucine-responsive regulatory protein; AAG, alkyl adenine glycosylase; AdoMet, *S*-adenosyl methionine

■ REFERENCES

(1) Halford, S. E. (2009) An end to 40 years of mistakes in DNA-protein association kinetics? *Biochem. Soc. Trans. 37*, 343−348.

(2) Halford, S. E., and Marko, J. F. (2004) How do site-specific DNA-binding proteins find their targets? *Nucleic Acids Res. 32*, 3040−3052.

(3) Pollak, A. J., Chin, A. T., Brown, F. L. H., and Reich, N. O. (2014) DNA looping provides for "intersegmental hopping" by proteins: a mechanism for long-range site localization. *J. Mol. Biol. 426*, 3539−3552.

(4) Horton, J. R., Liebert, K., Hattman, S., Jeltsch, A., and Cheng, X. (2005) Transition from nonspecific to specific DNA interactions along the substrate-recognition pathway of dam methyltransferase. *Cell 121*, 349−361.

(5) Vuzman, D., Azia, A., and Levy, Y. (2010) Searching DNA via a "monkey bar" mechanism: The significance of disordered tails. *J. Mol. Biol. 396*, 674−684.

(6) Doucleft, M., and Clore, G. M. (2008) Global jumping and domain-specific intersegmental transfer between DNA cognate site of the same multidomain transcription factore Oct-1. *Proc. Natl. Acad. Sci. U.S.A. 105*, 13871−13876.

(7) Terry, B. J., Jack, W. E., and Modrich, P. (1985) Facilitated diffusion during catalysis by EcoRI endonuclease. Non-specific interactions in EcoRI catalysis. *J. Biol. Chem. 260*, 13130−13137.

(8) Rau, D. C., and Sidorova, N. Y. (2010) Diffusion of the restriction nuclease EcoRI along DNA. *J. Mol. Biol. 395*, 408−416.

(9) Stanford, N. P., Szczelkun, M. D., Marko, J. F., and Halford, S. E. (2000) One- and three-dimensional pathways for proteins to reach specific DNA sites. *EMBO J. 19*, 6546−6557.

(10) Hedglin, M., and O'Brien, P. J. (2010) Hopping enables a DNA repair glycosylase to search both strands and bypass a bound protein. *ACS Chem. Biol. 5*, 427−436.

(11) Porecha, R. H., and Stivers, J. T. (2008) Uracil DNA glycosylase uses DNA hopping and short-range sliding to trap extrahelical uracils. *Proc. Natl. Acad. Sci. U.S.A. 105*, 10791−10796.

(12) Mendoza-Vargas, A., et al. (2009) Genome-wide identification of transcription start sites, promoters and transcription factor binding sites in *E. coli*. *PLoS One 4*, e7526.

(13) Casadesús, J., and Low, D. (2006) Epigenetic gene regulation in the bacterial world. *Microbiol. Mol. Biol. Rev. 70*, 830−856.

(14) Riggs, A. D., Bourgeois, S., and Cohn, M. (1970) The lac represser-operator interaction: III. Kinetic studies. *J. Mol. Biol. 53*, 401−417.

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

(15) Azam, T. A., Iwata, A., Nishimura, A., Ueda, S., and Ishihama, A. (1999) Growth phase-dependent variation in protein composition of the Escherichia coli nucleoid. *J. Bacteriol. 181*, 6361−6370.
(16) Luijsterburg, M. S., Noom, M. C., Wuite, G. J., and Dame, R. T. (2006) The architectural role of nucleoid-associated proteins in the organization of bacterial chromatin: a molecular perspective. *J. Struct. Biol. 156*, 262−272.
(17) Dillon, S. C., and Dorman, C. J. (2010) Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat. Rev. Microbiol. 8*, 185−195.
(18) Peterson, S. N., Dahlquist, F. W., and Reich, N. O. (2007) The role of high affinity non-specific DNA binding by Lrp in transcriptional regulation and DNA organization. *J. Mol. Biol. 369*, 1307−1317.
(19) Flyvbjerg, H., Keatch, S. A., and Dryden, D. T. (2006) Strong physical constraints on sequence-specific target location by proteins on DNA molecules. *Nucleic Acids Res. 34*, 2550−2557.
(20) Koslover, E. F., Díaz de La Rosa, M. A., and Spakowitz, A. J. (2011) Theoretical and computational modeling of target-site search kinetics in vitro and in vivo. *Biophys. J. 101*, 856−865.
(21) Li, G. W., Berg, O. G., and Elf, J. (2009) Effect of macromolecular crowding and DNA looping on gene regulation kinetics. *Nat. Phys. 5*, 294−297.
(22) Gorman, J., Plys, A. J., Visnapuu, M. L., Alani, E., and Greene, E. C. (2010) Visualizing one-dimensional diffusion of eukaryotic DNA repair factors along a chromatin lattice. *Nat. Struct. Mol. Biol. 17*, 932−938.
(23) Marcovitz, A., and Levy, Y. (2013) Obstacles may facilitate and direct DNA search by proteins. *Biophys. J. 104*, 2042−2050.
(24) Benichou, O., Chevalier, C., Meyer, B., and Voituriez, R. (2011) Facilitated diffusion of proteins on chromatin. *Phys. Rev. Lett. 106*, 038102.
(25) Baumann, C. G., Smith, S. B., Bloomfield, V. A., and Bustamante, C. (1997) Ionic effects on the elasticity of single DNA molecules. *Proc. Natl. Acad. Sci. U.S.A. 94*, 6185−6190.
(26) Shore, D., Langowski, J., and Baldwin, R. L. (1981) DNA flexibility studied by covalent closure of short fragments into circles. *Proc. Natl. Acad. Sci. U.S.A. 78*, 4833−4837.
(27) Ringrose, L., and Chabanis, S. (1999) Quantitative comparison of DNA looping in vitro and in vivo: chromatin increases effective DNA flexibility at short distances. *EMBO J. 18*, 6630−6641.
(28) Gowers, D. M., and Halford, S. E. (2003) Protein motion from non-specific to specific DNA by three-dimensional routes aided by supercoiling. *EMBO. J. 22*, 1410−1418.
(29) van den Broek, B., Lomholt, M. A., Kalisch, S. M. J., Metzler, R., and Wuite, G. J. L. (2008) How DNA coiling enhances target localization by proteins. *Proc. Natl. Acad. Sci. U.S.A. 105*, 1578−15742.
(30) Sidorova, N. Y., Scott, T., and Rau, D. C. (2013) DNA concentration dependent dissociation of EcoRI: direct transfer or reaction during hopping. *Biophys. J. 104*, 1296−13039.
(31) Hedglin, M., Zhang, Y., and O'Brien, P. J. (2013) Isololating contributions from intersegmental transfer to DNA searching by Alkyladenine DNA Glycosylase. *J. Biol. Chem. 288*, 24550−24559.
(32) Wentzell, L. M., and Halford, S. E. (1998) DNA looping by the SfiI restriction endonuclease. *J. Mol. Biol. 281*, 433−444.
(33) Mashhoon, N., Carroll, M., Pruss, C., Eberhard, J., Ishikawa, S., Estabrook, R. A., and Reich, N. O. (2004) Functional characterization of *Escherichia coli* DNA adenine methyltransferase, a novel target for antibiotics. *J. Biol. Chem. 279*, 52075−52081.
(34) Wion, D., and Casadesus, J. (2006) N^6-methyl-adenine: an epigenetic signal for DNA-protein interactions. *Nat. Rev. Microbiol. 4*, 183−192.
(35) Kunkel, T. A., and Erie, D. A. (2005) DNA Mismatch Repair. *Annu. Rev. Biochem. 74*, 681−710.
(36) Pluciennik, A., and Modrich, P. (2007) Protein roadblocks and helix discontinuities are barriers to the initiation of mismatch repair. *Proc. Natl. Acad. Sci. U.S.A. 104*, 12709−12713.
(37) Pollak, A. J., and Reich, N. O. (2012) Proximal recognition sites facilitate intrasite hopping by DNA adenine methyltransferase: mechanistic exploration of epigenetic gene regulation. *J. Biol. Chem. 287*, 22873−22881.
(38) Jeltsch, A., Alves, J., Wolfes, H., Maass, G., and Pingoud, A. (1994) Pausing of the restriction endonuclease EcoRI during linear diffusion on DNA. *Biochemistry 33*, 10215−10219.
(39) Martin, A. M., Horton, N. C., Lusetti, S., Reich, N. O., and Perona, J. J. (1999) Divalent metal dependence of site-specific DNA binding by Eco RV endonuclease. *Biochemistry 38*, 8430−8439.
(40) Winkler, et al. (1993) The crystal structure of EcoRV endonuclease and of its complexes with cognate and non-cognate DNA fragments. *EMBO J. 12*, 1781−1795.
(41) Hiller, D. A., Fogg, J. M., Martin, A. M., Beechem, J. M., Reich, N. O., and Perona, J. J. (2003) Simultaneous DNA binding and bending by EcoRV endonuclease observed by real-time fluorescence. *Biochemistry 42*, 14375−14385.
(42) Baldwin, G. S., Sessions, R. B., Erskine, S. G., and Halford, S. E. (1999) DNA cleavage by the EcoRV restriction endonucleases: roles of divalent metal ion in specificity and catalysis. *J. Mol. Biol. 288*, 87−103.
(43) Fersht, A. (1998) *Structure and Mechanism in Protein Science*; W. H. Freeman, New York.
(44) Horton, N. C., Newberry, K. J., and Perona, J. J. (1998) Metal ion-mediated substrate-assisted catalysis in type II restriction endonucleases. *Proc. Natl. Acad. Sci. U.S.A. 95*, 13489−13494.
(45) Zinkel, S. S., and Crothers, D. M. (1987) DNA bend direction by phase sensitive detection. *Nature 328*, 178−181.
(46) Kahn, J. D., and Crothers, D. M. (1992) Protein-induced bending and DNA cyclization. *Proc. Natl. Acad. Sci. U.S.A. 89*, 6343−6347.
(47) van Dijk, M., and Bonvin, A. M. (2009) 3D-DART: a DNA structure modelling server. *Nucleic Acids Res. 37* (Web Server Issue), W235−W239 DOI: 10.1093/nar/gkp287.
(48) De Vries, S. J., van Dijk, M., and Bonvin, A. M. (2010) The HADDOCK web server for data-driven biomolecular docking. *Nat. Protoc. 5*, 883−897.
(49) Kim, S., et al. (2010) Probing allostery through DNA. *Science 339*, 816−819.
(50) Peterson, S. N., and Reich, N. O. (2008) Competitive Lrp and Dam Assembly at the pap Regulatory Region: Implications for Mechanisms of Epigenetic Regulation. *J. Mol. Biol. 383*, 92−105.
(51) Pollak, A. J., Chin, A. T., and Reich, N. O. (2014) Distinct facilitated diffusion mechanisms by *E. coli* Type II restriction endonucleases. *Biochemistry 53*, 7028−7037.
(52) de los Rios, S., and Perona, J. J. (2007) Structure of the *Escherichia coli* Leucine-responsive regulatory protein Lrp reveals a novel octameric assembly. *J. Mol. Biol. 366*, 1589−1602.
(53) Shrivastava, T., and Ramachandran, R. (2007) Mechanistic insights from the crystal structures of a feast/famine regulatory protein from Mycobacterium tuberculosis H37Rv. *Nucleic Acids Res. 35*, 7324−7335.
(54) Tapias, A., López, G., and Ayora, S. (2000) Bacillus subtilis LrpC is a sequence-independent DNA-binding and DNA-bending protein which bridges DNA. *Nucleic Acids Res. 28*, 552−559.
(55) Peterson, S. N., and Reich, N. O. (2006) GATC Flanking Sequences Regulate Dam Activity: Evidence for how Dam Specificity may Influence pap Expression. *J. Mol. Biol. 355*, 459−472.
(56) Gorman, J., and Greene, E. C. (2008) Visualizing one-dimensional diffusion of proteins along DNA. *Nat. Struct. Mol. Biol. 15*, 768−774.
(57) Gowers, D. M., Wilson, G. G., and Halford, S. E. (2005) Measurement of the contributions of 1D and 3D pathways to the translocation of a protein along DNA. *Proc. Natl. Acad. Sci. U.S.A. 102*, 15883−15888.
(58) Schonhoft, J. D., and Stivers, J. T. (2012) Timing facilitated site transfer of an enzyme on DNA. *Nat. Chem. Biol. 8*, 205−210.
(59) Dorman, C. J. (2004) H-NS: a universal regulator for a dynamic genome. *Nat. Rev. Microbiol. 2*, 391−400.

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192

(60) Liu, Q., and Richardson, C. C. (1993) Gene 5.5 protein of bacteriophage T7 inhibits the nucleoid protein H-NS of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A. 90*, 1761−1765.
(61) Ghaemmaghami, S., et al. (2003) Global analysis of protein expression in yeast. *Nature 425*, 737−741.

DOI: 10.1021/bi501344r
Biochemistry 2015, 54, 2181−2192