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Visualizing one-dimensional diffusion of eukaryotic DNA repair factors along a chromatin lattice

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Abstract

DNA-binding proteins survey genomes for targets using facilitated diffusion, which typically includes a one-dimensional (1D) scanning component for sampling local regions. Eukaryotic proteins must accomplish this task while navigating through chromatin. Yet it is unknown whether nucleosomes disrupt 1D scanning or eukaryotic DNA-binding factors can circumnavigate nucleosomes without falling off DNA. Here we use single-molecule microscopy in conjunction with nanofabricated curtains of DNA to show that the postreplicative mismatch repair protein complex Mlh1–Pms1 diffuses in 1D along DNA via a hopping/stepping mechanism and readily bypasses nucleosomes. This is the first experimental demonstration that a passively diffusing protein can traverse stationary obstacles. In contrast, Msh2–Msh6, a mismatch repair protein complex that slides while maintaining continuous contact with DNA, experiences a boundary upon encountering nucleosomes. These differences reveal important mechanistic constraints affecting intranuclear trafficking of DNA-binding proteins.

Virtually all DNA-binding proteins must use some form of facilitated diffusion (for example, hopping, jumping, sliding and/or intersegmental transfer) to scan the genome and locate targets^{1–4}. The advent of single-molecule imaging has led to a resurgence of interest in facilitated diffusion, and an emerging consensus agrees that many proteins can scan DNA via one-dimensional (1D) diffusion, where the proteins undergo a random walk while moving laterally along the helix^{2–7}. However, all of these studies have been limited to naked DNA substrates, which do not resemble the crowded environments that would be encountered *in vivo*, leaving the role of 1D diffusion in question under physiologically relevant settings^{3,4,7}. In eukaryotes, these processes must occur within the context of chromatin, which has the

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AUTHOR CONTRIBUTIONS

J.G. designed the TIRFM experiments and collected and analyzed the single-molecule data; A.J.P. designed and engineered all constructs, expressed and purified the proteins, did all ensemble-level characterization and conducted the immunoprecipitation experiments; M.-L.V. made and characterized all chromatin substrates; J.G., A.J.P., M.-L.V., E.A. and E.C.G. discussed the data and cowrote the paper.

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potential to hinder protein mobility^{3,4,7,8}. Motor proteins, such as RNA polymerase and other DNA translocases, solve this problem by using the chemomechanical energy derived from nucleotide hydrolysis to push their way through nucleosome obstacles^{9,10}. However, most DNA-binding proteins, such as transcription factors or DNA-repair proteins, cannot mechanically disrupt nucleosomes; therefore, other mechanisms must come into play if these proteins are to scan chromatin. Whether proteins can circumnavigate nucleosomes without dissociating from DNA remains an unresolved issue with direct bearing on how all eukaryotic DNA-binding proteins are trafficked throughout the nucleus^{3,4,7,8}. This problem led us to ask whether eukaryotic proteins that diffuse in 1D along DNA could circumnavigate individual nucleosomes and travel along nucleosomal arrays, and if so, what mechanistic principles affect mobility along chromatin.

We chose the *Saccharomyces cerevisiae* post-replicative mismatch repair (MMR) protein complexes Msh2–Msh6 and Mlh1–Pms1 as model systems for studying the physical basis of facilitated diffusion. MMR is a ubiquitous repair pathway that corrects errors (mismatches and small insertion/deletion loops) left behind by the replication machinery^{11–13}. Defects in MMR lead to elevated mutation rates, are linked to hereditary nonpolyposis colon cancer (HNPCC) and are associated with many sporadic tumors¹². Msh2–Msh6 and Mlh1–Pms1 are DNA-binding proteins required for MMR. During MMR, Msh2–Msh6 must locate lesions and also helps identify nearby signals differentiating parental and nascent DNA strands, whereas Mlh1–Pms1 must locate lesion-bound Msh2–Msh6 and then coordinates downstream steps in the reaction. Although Msh2–Msh6 and Mlh1–Pms1 are both ATPases, neither uses ATP for generating chemomechanical force; rather, nucleotide binding and hydrolysis are thought to serve as signaling mechanisms for coordinating the various stages of repair by regulating protein-protein interactions in the case of Mlh1–Pms1 or protein-DNA interactions with Msh2–Msh6^{11–13}. These or closely related protein complexes are also involved in mitotic and meiotic recombination, triplet-repeat expansion, class-switch recombination, somatic hypermutation and DNA-damage signaling checkpoints¹¹. All known functions of Msh2–Msh6 and Mlh1–Pms1 require targeting to specific structures within the genome, and the later stages of the MMR reaction involved in strand discrimination are also thought to involve 1D movement along DNA^{11–13}, making these protein complexes good candidates as model systems for single-molecule studies of facilitated diffusion.

RESULTS

Experimental approach for visualizing protein-DNA interactions

Using total internal reflection fluorescent microscopy (TIRFM) we have previously shown that Msh2–Msh6 moves on DNA via a sliding mechanism consistent with a model where it tracks the phosphate backbone¹⁴. To determine whether Mlh1–Pms1 also moves on DNA, we engineered the proteins (Fig. 1) with epitope tags (Flag and/or hemagglutinin) and labeled them with antibody-coupled quantum dots (QDs; Supplementary Methods). Gel shift and nitrocellulose filter-binding assays confirmed labeling specificity and showed that labeling did not disrupt DNA binding activity (Fig. 2a–c and Supplementary Methods). For TIRFM, we used microfluidic devices with hybrid surfaces comprised of fluid lipid bilayers and nanofabricated metallic barrier patterns made by electron-beam lithography¹⁵. The DNA substrates (λ -DNA, 48,502 base pairs) were anchored by one end to the bilayer through a biotin-streptavidin linkage, and hydrodynamic force was then used to push the DNA and align it along the leading edges of nanofabricated barriers to lipid diffusion¹⁵ (Fig. 1b). The second end of the DNA was then anchored to antibody-coated pentagons positioned downstream from the linear barriers¹⁵ (Fig. 1b). This strategy yields ‘double-tethered’ curtains of DNA in which the individual DNA molecules are suspended above a lipid bilayer and are anchored by both ends such that they can be viewed across their entire contour length by TIRFM in the absence of a

perturbing hydrodynamic flow (Fig. 1b,c, Supplementary Fig. 1, Supplementary Video 1 and Supplementary Methods).

Mlh1–Pms1 diffuses on DNA by a stepping or hopping mechanism

When imaged by TIRFM, Mlh1–Pms1 colocalized with DNA (Fig. 1c), and $\geq 95\%$ of the DNA-bound proteins moved rapidly back and forth along the DNA molecules (Fig. 1d, Supplementary Video 2 and Supplementary Table 1). Two-color labeling experiments revealed that most (98.4%) of the complexes were single heterodimers under the conditions used for these experiments (Supplementary Methods). Mlh1–Pms1 often remained bound to the DNA for several minutes without dissociating (Fig. 1c), consistent with bulk biochemical studies¹⁶. Analysis of the motion revealed linear mean-squared displacement (MSD) plots, as expected for 1D diffusion^{5,14} (Fig. 3a), yielding a mean diffusion coefficient of $D_{1D} = 0.143 \pm 0.29 \mu\text{m}^2 \text{sec}^{-1}$ ($N = 25$) at 150 mM NaCl, 1 mM ATP and 1 mM MgCl_2 (Fig. 3b). We observed 1D diffusion with or without ADP, ATP, and $\text{ATP}\gamma\text{S}$ (Fig. 3b), and the differences in the diffusion coefficients measured in the presence and absence of ADP or ATP were statistically insignificant (Student's t -test, $P \geq 0.01$). These results indicate that nucleotide binding and hydrolysis were unnecessary for movement, consistent with the notion that nucleotide binding is primarily involved in promoting protein-protein interactions or structural rearrangements, with little impact on DNA-binding^{11,13,17}.

Mlh1–Pms1 diffusion coefficients were an order of magnitude greater (Student's t -test, $P < 0.0001$) than those of Msh2–Msh6 under physiological salt concentrations ($0.143 \pm 0.29 \mu\text{m}^2 \text{sec}^{-1}$ vs. $0.009 \pm 0.011 \mu\text{m}^2 \text{sec}^{-1}$ at 150 mM NaCl; Fig. 3b)¹⁴, suggesting that the two complexes might move via different mechanisms. Potential mechanisms for diffusive motion along DNA include hopping, jumping, sliding or intersegmental transfer. The structure of Mlh1–Pms1 (refs. 17–20) (Fig. 1a) also suggested a possible ‘stepping’ mechanism, which is virtually identical to hopping, with the N- and/or C-terminal domains (NTD and CTD, respectively) acting as DNA-binding domains that independently hop while connected by flexible linkers. Jumping would yield punctate kymograms as a consequence of repeated dissociation and rebinding events and cannot account for the continuous motion that predominated the diffusion trajectories; the stretched DNA configuration makes intersegmental transfer involving DNA-looping unlikely; and the 38-fold increase (Student's t -test, $P < 0.0001$) in the diffusion coefficient measured over a range of salt concentrations argues against sliding ($D_{1D} = 0.026 \pm 0.017 \mu\text{m}^2 \text{sec}^{-1}$ versus $0.99 \pm 0.411 \mu\text{m}^2 \text{sec}^{-1}$, at 25 and 200 mM NaCl, respectively; Fig. 3c and Supplementary Fig. 2) but is consistent with a hopping and/or stepping mechanism (see Supplementary Discussion)^{5,21}. In contrast to Mlh1–Pms1, we have previously shown that the diffusion coefficient of Msh2–Msh6 does not vary over the same range of NaCl concentrations (see Supplementary Fig. 3a from ref. 14), which is most consistent with a sliding mechanism¹⁴. We conclude that, although Msh2–Msh6 and Mlh1–Pms1 both travel along DNA via 1D diffusion, they do so using different mechanisms: Msh2–Msh6 slides while in continuous contact with the phosphate backbone, whereas Mlh1–Pms1 hops or steps as it moves back and forth along DNA.

Mlh1–Pms1 has properties consistent with ring-like architecture

The Mlh1–Pms1 heterodimer is maintained through protein-protein interactions between the CTDs, and the NTDs and CTDs are separated by very long linker arms^{17,22} (Fig. 1a). It has been previously hypothesized that this structural organization allows Mlh1–Pms1 and related proteins to adopt a ring-like architecture, which would enable them to encircle DNA¹⁹ (Fig. 1a). This type of topological binding mechanism leads to several specific experimentally testable predictions: (i) dissociation from DNA should occur preferentially from the free ends of linear DNA molecules; (ii) protein dissociation should be prevented if the DNA ends are sterically occluded; (iii) dissociation should be less prevalent from internal positions; and (iv)

an intact heterodimer would be necessary for stable DNA-binding activity and end-dependent dissociation. We first asked whether Mlh1–Pms1 preferentially dissociated from DNA ends (Fig. 4a–d). When hydrodynamic force (~100 fN; Supplementary Methods) was used to push Mlh1–Pms1, most complexes (>95%) did not dissociate upon encountering anchored (that is, sterically blocked) DNA ends (Fig. 4a; $N_{\text{dis}}/N_{\text{tot}} = 1/23$ (dissociated / total pushed to DNA ends)), nor did Mlh1–Pms1 dissociate from the apex of looped DNA (Fig. 4d; $N_{\text{dis}}/N_{\text{tot}} = 0/4$). In contrast, Mlh1–Pms1 immediately dissociated from free ends of ‘single-tethered’ DNA (Fig. 4b; $N_{\text{dis}}/N_{\text{tot}} = 880/1,000$) and from free ends of photochemically induced double-stranded breaks (DSBs; Fig. 4c,d; $N_{\text{dis}}/N_{\text{tot}} = 14/14$). Mlh1 alone can exist as monomers or dimers ($K_d = 3.14 \pm 0.19 \mu\text{M}$), but the Mlh1-NTDs do not self associate²². Mlh1 alone could bind DNA (Fig. 4e), with a diffusion coefficient 6.9-fold greater ($P < 0.0001$) than that of Mlh1–Pms1 under the same conditions ($D_{1D} = 0.137 \pm 0.127 \mu\text{m}^2 \text{sec}^{-1}$, $N = 25$, versus $0.020 \pm 0.023 \mu\text{m}^2 \text{sec}^{-1}$, $N = 25$, respectively, at 50 mM NaCl; we did not detect Mlh1 binding at higher ionic strengths), indicating that Pms1 was not essential for binding or diffusion. However, when flow was applied, Mlh1 moved rapidly down the DNA, and >80% dissociated from the anchored DNA ends (Fig. 4e; $N_{\text{dis}}/N_{\text{tot}} = 285/350$). This finding was strikingly different from results with the intact heterodimer, indicating that the presence of Pms1 was necessary to observe end-dependent dissociation. Finally, we engineered TEV cleavage sites into the linker of Mlh1 and Pms1, and proteolytic cleavage of one or both linker arms abolished detectable DNA-binding activity (Supplementary Fig. 3), highlighting the importance of the linker arms for DNA binding. We conclude that the formation of an intact Mlh1–Pms1 heterodimer stabilizes the DNA-bound complex and that the heterodimer preferentially dissociates from DNA ends. These experimental findings are all consistent with predictions for the previously proposed mechanism whereby Mlh1–Pms1 can adopt a ring-like architecture that wraps around DNA, although we are careful to note that we do not yet know the structural details of the wrapped complex.

Mlh1–Pms1 can bypass one another while traveling along DNA

We have previously shown that Msh2–Msh6 complexes traveling on the same molecule cannot pass one another, arguing that the proteins maintain continuous close contact with the DNA, which is consistent with a sliding mechanism¹⁴. In contrast, two-color labeling experiments revealed that Mlh1–Pms1 complexes could bypass one another as they traveled along the same DNA molecule (Supplementary Fig. 4), which is only consistent with a hopping/stepping mechanism wherein the individual hops or steps span distances comparable to or greater than the dimensions of the QD-tagged proteins. Closed ring-like architecture is difficult to reconcile with the observed protein bypass and would require two Mlh1–Pms1 complexes to thread through one another as they moved along the DNA. A threading mechanism specifically predicts that Mlh1–Pms1 would be unable to bypass obstacles larger than the internal diameter of the large central pore formed by the protein complex. Alternatively, bypass could also be accomplished through transient ring opening, whereupon the proteins could simply step past one another in an open configuration. This type of open stepping mechanism predicts that Mlh1–Pms1 would be capable of bypassing obstacles larger than the internal diameter of the protein ring. Given the combined length of the Mlh1–Pms1 linker arms ($51.7 \pm 14.6 \text{ nm}$)¹⁷, the corresponding maximal diameter of the central pore would be $16.5 \pm 4.6 \text{ nm}$ in diameter, which is too small to accommodate the passage of a QD (~20 nm in diameter), ruling out a threading mechanism for obstacle bypass. We conclude that Mlh1–Pms1 most likely bypasses obstacles by stepping over them in an open ring configuration, implying that the protein is capable of transitioning back and forth between an open and closed conformation.

Mlh1–Pms1 can traverse nucleosomes during 1D diffusion

The finding that Mlh1–Pms1 complexes could bypass one another suggested that these proteins might be able to undergo 1D diffusion on crowded DNA substrates, similar to what would be

found in an *in vivo* environment. Therefore, we next asked whether MMR proteins could traverse nucleosomes, which are anticipated to be the most abundant obstacles encountered in eukaryotes. For these experiments, we deposited unlabeled, recombinant nucleosomes onto the DNA substrates by salt dialysis at a ratio of either ~5–10 or ~80–100 nucleosomes per DNA molecule²³. We performed the Mlh1–Pms1 diffusion measurements as described above, and we then located the nucleosomes by labeling them with QDs after the diffusion measurements were completed (Supplementary Methods). Mlh1–Pms1 still diffused on nucleosome-bound DNA ($D_{1D} = 0.027 \pm 0.021 \mu\text{m}^2 \text{sec}^{-1}$, $N = 26$) and repeatedly bypassed unlabeled nucleosomes (~10 nm in diameter), showing no evident boundary effects upon colliding with single nucleosomes (Fig. 5a, upper panel; $N > 1,000$ Mlh1–Pms1 complexes, each giving rise to multiple bypass events). Mlh1–Pms1 also moved freely along DNA bound by up to ~80–100 unlabeled nucleosomes (Fig. 5a, middle panel; $D_{1D} = 0.034 \pm 0.018 \mu\text{m}^2 \text{sec}^{-1}$, $N = 25$), providing an unequivocal demonstration that nucleosomes do not prevent 1D diffusion of Mlh1–Pms1. We conclude Mlh1–Pms1 can travel along a simple chromatin lattice by 1D diffusion while bypassing protein obstacles as it travels along the DNA. As indicated above, for all of these experiments, we labeled the nucleosomes only after making the diffusion measurements to ensure that the large QDs would not interfere with Mlh1–Pms1 movement. However, Mlh1–Pms1 could also bypass QD-labeled nucleosomes ($N = 63$ Mlh1–Pms1 complexes, each yielding multiple bypass events), although in this case, Mlh1–Pms1 showed characteristics of bounded diffusion upon colliding with the QD-labeled nucleosomes, with the large QD-labeled nucleosomes acting as semipenetrable barriers (Fig. 5a, lower panel). Given the large diameter of the QD-labeled-nucleosome (≥ 30 nm) compared to the size of Mlh1–Pms1, we conclude that nucleosome bypass must occur via a stepping mechanism whereby the protein transiently adopts an open ring configuration. These results provide the first experimental demonstration that a protein undergoing 1D diffusion can circumnavigate protein obstacles that lie in its path.

Diffusion of Msh2–Msh6 is restricted by nucleosomes

In striking contrast to Mlh1–Pms1, the movement of Msh2–Msh6 past unlabeled nucleosomes was highly restricted, showing characteristics of bounded diffusion with nucleosomes acting as semipenetrable barriers, and Msh2–Msh6 typically became trapped between nucleosomes (Fig. 5b, upper and middle panels). On higher-density nucleosome arrays (~80–100 nucleosomes per 48.5 kb DNA substrate) most molecules of Msh2–Msh6 were immobile or oscillated within tightly confined regions ($N = 964 / 1000$; Supplementary Methods) and showed little evidence of free 1D diffusion within our detection limits ($D_{1D} \leq 1 \times 10^{-4} \mu\text{m}^2 \text{sec}^{-1}$). A small subpopulation of Msh2–Msh6 remained mobile on the high-density arrays ($N = 36 / 1000$; 3.6%), suggesting that they were bound in an alternate conformation. In further contrast to Mlh1–Pms1, Msh2–Msh6 never bypassed QD-tagged nucleosomes (Fig. 5b, lower panel), indicating that large obstacles (≥ 30 nm in diameter) present insurmountable barriers, which is fully consistent with expectations based on the structure of Msh2–Msh6, which wraps around DNA, making intimate contacts with the phosphate backbone over nearly 1.5 turns of helix²⁴, and is also consistent with a continuous sliding mechanism that does not involve extensive hopping¹⁴. Rare nucleosome bypass by Msh2–Msh6 might occur through occasional hopping events or through limited excursions into two-dimensional sliding, where Msh2–Msh6 maintains contact with the DNA without tracking the helical pitch of the phosphate backbone^{6,8} (Fig. 5c). In either case, the mechanism does not permit efficient mobility of Msh2–Msh6 along the higher-density nucleosome arrays. This conclusion agrees with bulk biochemical studies showing that nucleosomes or other stationary obstacles can trap Msh2–Msh6 (or its homologs) on DNA^{25–28}.

DISCUSSION

Intranuclear trafficking of all DNA-binding proteins is governed by facilitated diffusion. Theoretical descriptions and bulk measurements of facilitated diffusion have long been reported in the literature, beginning with the classical studies of *lac* repressor^{29–32} and more recently with NMR experiments of transcription factors^{33–35}, but direct measurements of diffusion have only recently become possible through the development of new single-molecule techniques^{5,6,14,36–38}. Together, these studies support an emerging consensus that many DNA-binding proteins can travel long distances along DNA by 1D diffusion *in vitro*. However, the validity of this conclusion with respect to physiological settings remains unclear despite years of experimental and theoretical efforts, specifically because it remains unknown whether or how 1D diffusion can occur in the presence of nucleosomes and other nucleoprotein structures^{1,3,4,7,8,39,40}. Here we sought to resolve this issue by using single-molecule imaging, nanofabricated curtains of double-tethered DNA molecules, and MMR proteins as model systems for facilitated diffusion.

We have shown that both Msh2–Msh6 and Mlh1–Pms1 can diffuse in 1D along DNA but do so using very different mechanisms. Mlh1–Pms1 hops or steps along the DNA, but Msh2–Msh6 moves predominantly by sliding along the DNA while remaining in continuous close contact with the phosphate backbone. The functional consequences of these mechanistic differences are that Mlh1–Pms1 can readily traverse nucleosomes and travel along chromatin, whereas Msh2–Msh6 cannot. These results provide an unambiguous demonstration that 1D diffusion can occur on crowded DNA substrates in the presence of protein obstacles and that the ability to bypass obstacles is dependent on how the protein in question diffuses along DNA. We anticipate that these behaviors shown by Mlh1–Pms1 and Msh2–Msh6 in response to collisions with nucleosomes will reflect general mechanistic attributes of their respective modes of 1D diffusion, which will apply in principle to any proteins that diffuse on DNA (for example, DNA repair proteins, transcription factors, etc.). Proteins that track the phosphate backbone while sliding along DNA will experience a barrier upon encountering obstacles. Therefore either the protein must disengage the DNA and enter a two- or three-dimensional mode of diffusion to continue searching for targets or the DNA must be cleared of obstacles beforehand to allow unhindered access to the DNA (see below). In contrast, proteins that do not track the backbone can traverse obstacles without experiencing boundary effects.

The different modes of diffusion found for Msh2–Msh6 and Mlh1–Pms1 also impose specific constraints on the mechanisms of MMR. Msh2–Msh6 is the first to arrive at lesions, and helps identify nearby signals differentiating the parental and nascent DNA strands. Many models for strand discrimination invoke 1D movement of Msh2–Msh6 along DNA, and even transient loss of contact with the DNA during this second phase of the reaction could compromise repair^{11–13}. Nucleosomes, or other DNA-binding proteins, have the potential to thwart Msh2–Msh6, and a single nucleosome deposited near a lesion could render it irreparable, suggesting that regions in need of repair must be kept free from obstacles. Replication forks disrupt nucleosomes, leaving stretches of naked DNA in their wake⁴¹. Although it is speculative, if Msh2–Msh6 were restricted to the region behind the fork, possibly through direct association with PCNA, then it would be free to scan newly replicated, naked DNA^{14,42,43}. Mlh1–Pms1 is thought to arrive later than Msh2–Msh6 (refs. 11–13), implying that it must survey the entire genome for lesion-bound Msh2–Msh6 without the benefit of confined searches in regions already cleared by a replication fork. The ability of Mlh1–Pms1 to hop or step along DNA and to freely traverse nucleosomes ensures that it could efficiently bypass stationary obstacles while searching the genome for its binding targets.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/nsmb/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Nanofabricated racks of DNA for visualizing 1D diffusion of Mlh1-Pms1. **(a)** Illustration of some predicted structures for Mlh1-Pms1 based on structural and biochemical data^{17–20}. The NTDs, CTDs, central pore and linker arms are indicated. **(b)** Diagram of the nanofabricated rack device used for making the double-tethered DNA curtains (Supplementary Methods)¹⁵. The rack consists of linear barriers to lipid diffusion, which align the lipid-tethered DNA molecules, followed by an array of antibody-coated pentagons that provide immobile anchor points for the second end of the DNA. Pattern elements are ~20 nm tall, and the bilayer is ~5 nm thick. **(c)** Top and bottom, YOYO1-stained λ-DNA curtains (green; 48,502 base pairs) with and without QD-tagged Mlh1-Pms1 (magenta), respectively (Supplementary Videos 1 and 2).

DNA-bound proteins were not detected in reactions using incorrect antibody-epitope pairs (not shown), and QDs alone did not bind DNA (not shown). **(d)** Kymograms illustrating the motion of Mlh1–Pms1. The lower panel shows photocleavage (arrowhead) of a DNA during data collection. The proteins disappear from view when the DNA breaks, showing that the proteins and DNA are not adsorbed to the bilayer.

Figure 2.

DNA-binding activity of fluorescently tagged Mlh1-Pms1. **(a)** Gel mobility shifts were performed as described in Online Methods. Reactions contained 120 nM Mlh1-Pms1 and 60 nM 5'-³²P-labeled 40-mer dsDNA. Antibodies were preincubated with Mlh1-Pms1, as indicated. **(b)** Nitrocellulose filter binding assays were performed with 100 pM 5'-³²P-labeled 3-kb linear plasmid at the indicated concentrations of Mlh1-Pms1, and the percentage of bound DNA was determined by dividing the background subtracted counts measured for each filter by the total amount of DNA in the reactions. **(c)** The effects of antibodies and antibody-labeled QDs on the binding activity of Mlh1-Pms1 (20 nM) as determined by the nitrocellulose filter-binding assay. All data points are reported as mean $\pm$ s.d.

Figure 3.

Quantitative analysis of Mlh1-Pms1 diffusion. **(a)** Examples of MSD plots generated from tracking the motion of Mlh1-Pms1. **(b)** Diffusion coefficients derived from the MSD plots and categorized according into the different nucleotide conditions used for the measurements. Diamonds ($\blacklozenge$), the mean values; the mean $\pm$ s.d. are color coded (graph and inset; $N \geq 25$ for all reported diffusion coefficients). The cumulative diffusion coefficients for Mlh1-Pms1 and the previously measured values for Msh2-Msh6 are shown together for comparison¹⁴, and the upper boundaries for the theoretical diffusion coefficients based on models either with or without a rotational component are also shown^{14,44}. **(c)** Diffusion coefficients for Mlh1-Pms1 determined at different concentrations of NaCl.

Figure 4.

End-dependent dissociation of Mlh1-Pms1 from DNA. **(a)** Kymogram of Mlh1-Pms1 (magenta) diffusing on a DNA molecule (unlabeled) anchored by both ends to the flow-cell surface. Flow is cycled on and off as indicated. When flow is on, Mlh1-Pms1 is pushed to the downstream anchored end of the DNA but does not dissociate. **(b)** Kymograms of Mlh1-Pms1 dissociating from the free blunt ends (generated by SfoI digest) of single-tethered DNA molecules. **(c)** Mlh1-Pms1 (magenta) was bound to DNA (green) stained with YOYO1 and was pushed repeatedly to the end of the molecule (as indicated) to verify that it did not dissociate. A DSB was introduced by laser illumination in the absence of flow. Upon breaking, the DNA retracts from the surface, as indicated by the sudden disappearance of the green signal.

Flow was resumed to extend the broken DNA and push Mlh1–Pms1 toward the free end of the molecule. Mlh1–Pms1 immediately dissociates upon encountering the free DNA end (see inset). **(d)** Mlh1–Pms1 was bound to a looped DNA molecule (i) and slid along the arc formed by the DNA until stopping at the loop apex (ii). The protein remained at the DNA apex (iii and iv) but continued sliding down the DNA upon induction of a DSB (v), and the protein immediately dissociated upon reaching the newly generated free end (vi), leaving behind the naked DNA (vii). **(e)** Kymogram of Mlh1 bound to a double-tethered DNA molecule. In the absence of flow the proteins diffuse rapidly along the DNA, but when flow is applied, they are pushed to the end of the DNA and rapidly dissociate. Experiments in **a–e** were collected from isolated DNA molecules, as described¹⁴. The DNA in **a** and **b** was located with YOYO1, but the dye was removed before data acquisition to avoid unintentional photocleavage, and the experiments were conducted at 150 mM NaCl. The experiments in **c**, **d** and **e** were conducted at 50 mM NaCl. In all cases, identical results were obtained ± 1 mM ATP.

Figure 5.

Diffusion of Mlh1-Pms1 and Msh2-Msh6 along nucleosome-bound DNA. **(a)** Mlh1-Pms1 (magenta) is shown diffusing along a DNA bound by recombinant nucleosomes (green); white, regions of overlapping signal. The nucleosomes were labeled with QDs either after conducting the Mlh1-Pms1 diffusion experiment (upper and middle panels) or before the addition of Mlh1-Pms1 (lower panel). Examples of bypass and bounded diffusion are highlighted; identical results were obtained $\pm$ ATP. **(b)** Msh2-Msh6 (red) is shown diffusing on a DNA molecule with unlabeled nucleosomes (green); yellow, regions of overlap. Upper and middle panels, results with unlabeled nucleosomes. The number designations (either 1 or 2) in the middle panel indicate number of QD-tagged Msh2-Msh6 molecules trapped in each region of

the kymogram (6 total). Lower panel, Msh2–Msh6 colliding with QD-labeled nucleosomes. See Supplementary Methods for additional experimental details. (c) Illustrates how the structures of Mlh1–Pms1 (left) and Msh2–Msh6 (right) may influence nucleosomal encounters^{18–20,24,45}. The molecules are drawn to scale. The trajectory of the DNA leaving the nucleosome surface has been modified for illustrative purposes. Mlh1–Pms1 steps over the nucleosome (solid arrow). Nucleosome bypass by Msh2–Msh6 might occur by occasional hopping or two-dimensional (2D) sliding (dashed arrows)⁸, but neither mechanism allows free mobility on a nucleosomal array.