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RecN motor activity drives RecA-mediated homology search in
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To my late mother, who dreamed of an education for her children that she never had;

to my father, whose hard work made that dream possible;

to my sister, whose love and affection have always comforted me;

and to my brother, whose example and guidance helped bring me here.

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Contribution statement

The research presented in this thesis was carried out at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), in Vienna, as part of the Vienna BioCenter (VBC) PhD program under the supervision of Dr. Anton Goloborodko. I was responsible for the theoretical development, calculations, simulations, analysis, interpretation, figure preparation, and writing of the thesis, unless explicitly stated otherwise.

All simulations and computational work presented in this thesis were performed by me, using computational resources provided by the CLIP cluster, and the Austrian Scientific Computing (ASC) infrastructure.

Therefore, the thesis reflects my theoretical and computational contribution to the project. Discussions with my supervisor and collaborators helped shape the broader scientific context of the work. All external contributions, materials, data, and previously published work have been acknowledged where relevant.

In accordance with the university's AI policy, I declare that standard spelling and grammar tools, as well as AI-based language models such as ChatGPT, were used during the preparation of this thesis to improve readability, clarity, and phrasing. These tools were used only for language-related support. They were not used to generate scientific content, design the methodology, produce or modify data, perform simulations or analyses, or draw conclusions. All scientific ideas, interpretations, results, and conclusions presented in this thesis are my own. No algorithmic or AI-based tool was used to fabricate, falsify, manipulate, or misrepresent any data, analyses, or results.

The Zusammenfassung was generated using DeepL as per university guidelines for non-German speakers.

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Abstract

DNA double-strand breaks (DSBs) are common lesions that arise during genome replication and other cellular processes. In bacteria, these breaks are repaired through homology-dependent repair (HDR), in which RecA forms a presynaptic nucleoprotein filament on the processed single-stranded DNA end of the break. This filament must search the bacterial genome for a homologous template to initiate strand invasion and repair. Previous studies showed that RecN, a bacterial SMC-like protein, is essential for efficient DSB repair in *Caulobacter crescentus*, where it promotes pole-to-pole translocation of the RecA filament and enhances homology search. However, the physical mechanism by which RecN drives this intracellular motion remains unclear.

Here, I propose that, akin to other SMCs, RecN may work as a DNA motor, and when bound to RecA filament, it may drive filament motion by transiently engaging nearby DNA. To test this hypothesis, I developed a coarse-grained polymer model where RecN is incorporated as a RecA filament bound DNA motor. The simulations show that a small number of RecN molecules can generate productive motion and accelerate homology search. The model further explains the necessity of the RecA filament rigidity for directional persistence, and predicts the microscopic arrangement of the filament and DNA from the two ends of the break site.

Finally, the model suggests that RecN-driven translocation may enhance homology search in other bacterial systems. In an *E. coli*-like model, RecN motor activity explains the experimental observations better than previously proposed models. In a spherical geometry it improves search efficiency primarily by enhancing filament mobility and genome exploration. Since RecA-mediated repair and RecN protein is broadly conserved, similar motor-assisted filament motion could provide a general strategy for improving genome-wide homology search, although species-specific chromosome organization and repair dynamics may modulate its effectiveness.

Zusammenfassung

DNA-Doppelstrangbrüche (DSBs) sind häufige Schäden, die während der Genomreplikation und anderer zellulärer Prozesse entstehen. In Bakterien werden diese Brüche durch homologe Reparatur (HDR) behoben, bei der RecA ein präsynaptisches Nukleoproteinfilament am aufbereiteten einzelsträngigen DNA-Ende des Bruchs bildet. Dieses Filament muss das bakterielle Genom nach einer homologen Matrize absuchen, um die Stranginvasion und die Reparatur einzuleiten. Frühere Studien zeigten, dass RecN, ein bakterielles SMC-ähnliches Protein, für eine effiziente DSB-Reparatur in *Caulobacter crescentus* essenziell ist, wo es die Pol-zu-Pol-Translokation des RecA-Filaments fördert und die Homologiesuche verbessert. Der physikalische Mechanismus, durch den RecN diese intrazelluläre Bewegung antreibt, bleibt jedoch unklar.

Hier stelle ich die Hypothese auf, dass RecN, ähnlich wie andere SMCs, als DNA-Motor fungieren könnte und, wenn es an ein RecA-Filament gebunden ist, die Filamentbewegung antreibt, indem es vorübergehend an benachbarte DNA andockt. Um diese Hypothese zu überprüfen, habe ich ein grobkörniges Polymermodell entwickelt, in das RecN als an ein RecA-Filament gebundener DNA-Motor integriert ist. Die Simulationen zeigen, dass bereits eine geringe Anzahl von RecN-Molekülen eine produktive Bewegung erzeugen und die Homologiesuche beschleunigen kann. Das Modell erklärt zudem die Notwendigkeit der Steifigkeit des RecA-Filaments für die Richtungsbeständigkeit und sagt die mikroskopische Anordnung des Filaments und der DNA an den beiden Enden der Bruchstelle voraus.

Schließlich legt das Modell nahe, dass die durch RecN gesteuerte Translokation die Homologiesuche in anderen bakteriellen Systemen verbessern könnte. In einem *E. coli*-ähnlichen Modell erklärt die Motoraktivität von RecN die experimentellen Beobachtungen besser als bisher vorgeschlagene Modelle. In einer sphärischen Geometrie verbessert sie die Suche effizienz in erster Linie durch die Steigerung der Filamentmobilität und der Genomerkundung. Da die RecA-vermittelte Reparatur und das RecN-Protein weitgehend konserviert sind, könnte eine ähnliche motorunterstützte Filamentbewegung eine allgemeine Strategie zur Verbesserung der genomweiten Homologiesuche darstellen, auch wenn artspezifische Chromosomenorganisation und Reparaturdynamik ihre Wirksamkeit beeinflussen könnten.

1. Introduction

Deoxyribonucleic acid (DNA) encodes the genetic information required for development, growth, cellular function, and reproduction in most organisms (Alberts, 2017). Damage to DNA can compromise any of these essential processes and, if unrepaired, may be lethal. Such damage can result in mutations within protein-coding sequences, disruptions in gene regulation, impaired development and cell division, oncogenic transformation, or cell death. DNA lesions arise from a wide range of cellular processes and environmental mutagens and are broadly classified as endogenous or exogenous based on their origin (Bouwman & Crosetto, 2018; Chatterjee & Walker, 2017; Ciccia & Elledge, 2010; Hoeijmakers, 2009; Lindahl & Barnes, 2000).

Endogenous DNA damage primarily arises as an unavoidable consequence of normal cellular metabolism (Fig. 1.1). Reactive oxygen species (ROS), generated during oxidative phosphorylation and other metabolic reactions, can oxidize DNA bases, leading to lesions such as 8-oxoguanine that promote mutagenic base mispairing (Cooke et al., 2003; Hoeijmakers, 2009; Lindahl, 1993). Replication stress, caused by nucleotide depletion, DNA secondary structures, or conflicts between replication and transcription, can result in stalled or collapsed replication forks, generating single-strand breaks (SSBs) or double-strand breaks (DSBs) (Bouwman & Crosetto, 2018; Zeman & Cimprich, 2014). Additionally, spontaneous chemical reactions such as base deamination, depurination, and methylation can alter nucleotide identity or destabilize the DNA backbone, introducing point mutations or abasic sites that threaten genome integrity (Lindahl, 1993; Lindahl & Barnes, 2000).

Exogenous DNA damage originates from environmental and chemical agents (Fig. 1.1) (Bouwman & Crosetto, 2018; Desouky et al., 2015). Ultraviolet (UV) radiation induces the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts, which distort the DNA helix and impede transcription and replication (Cadet et al., 2005). Ionizing radiation generates a spectrum of lesions, including base damage, SSBs, and highly cytotoxic DSBs, often accompanied by clustered damage sites that are difficult to repair. Chemical mutagens such as alkylating agents modify DNA bases and phosphodiester backbones, leading to mispairing or strand breaks, while environmental toxins and chemotherapeutic drugs can form bulky DNA adducts or interstrand crosslinks that block DNA processing enzymes (Chatterjee & Walker, 2017; Friedberg et al., 1995). Collectively, these diverse forms of DNA damage necessitate robust and tightly regulated DNA repair pathways to preserve genomic stability.

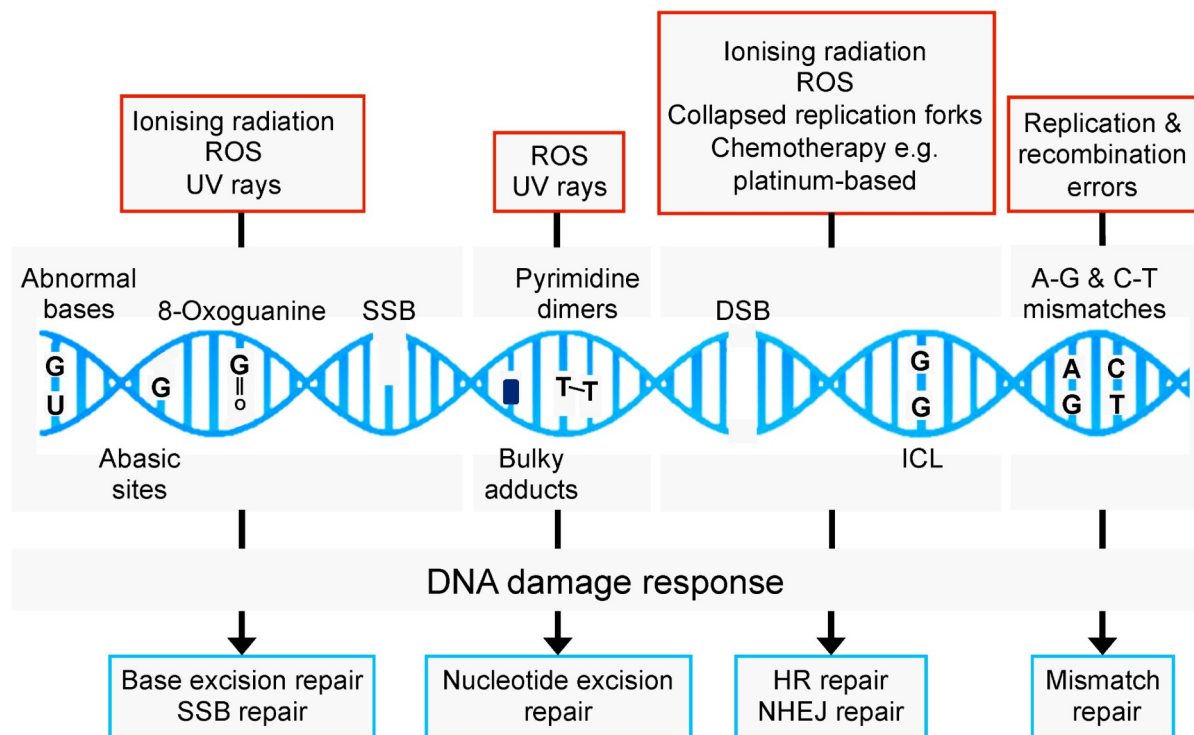


Figure 1.1: Common causes of endogenous and exogenous DNA damage and their repair pathways. HR: homologous recombination; NHEJ: non-homologous end-joining; SSB: single-strand break; BER: base excision repair; ICL: interstrand crosslink. This figure is adapted from (Gillespie et al., 2023).

Among the various forms of DNA damage, double-strand breaks (DSBs) are considered one of the most severe and cytotoxic lesions (Jackson & Bartek, 2009; Khanna & Jackson, 2001). DSBs involve the simultaneous breakage of both strands of the DNA duplex, resulting in the physical discontinuity of the chromosome. Such lesions can arise from endogenous processes, including replication fork collapse, oxidative stress, and programmed DNA cleavage events, as well as from exogenous sources such as ionizing radiation and genotoxic chemicals (Bouwman & Crosetto, 2018; Chatterjee & Walker, 2017; Hoeijmakers, 2009; Jackson & Bartek, 2009; Khanna & Jackson, 2001; Kuzminov, 2001). If left unrepaired or misrepaired, DSBs can lead to chromosomal fragmentation, large-scale deletions, translocations, and loss of genetic material, ultimately threatening cell viability and genomic stability (Chapman et al., 2012; Ciccia & Elledge, 2010).

Quantitative estimates indicate that double-strand breaks occur at appreciable frequencies across all domains of life, even under normal growth conditions (Bouwman & Crosetto, 2018; Vilenchik & Knudson, 2003). In bacteria such as *Escherichia coli*, spontaneous double-strand breaks occur at low but significant frequencies during normal bacterial growth, primarily as a consequence of replication fork collapse (Kuzminov, 1999). In budding yeast (*Saccharomyces cerevisiae*), genome-wide measurements suggest the formation of at least one DSB in a few

cell divisions, originating from replication-associated damage, transcription-replication conflicts, and endogenous metabolic byproducts (Haber, 2018; Lisby et al., 2001). In mammalian cells, the burden is even higher: proliferating human cells are estimated to experience 10-50 DSBs per cell per day (Bouwman & Crosetto, 2018; Vilenchik & Knudson, 2003), while exposure to ionizing radiation can induce dozens of additional breaks per gray of radiation dose. These measurements underscore that DSBs are not rare catastrophic events, but rather recurrent challenges that cells must continuously detect and repair to maintain genome integrity.

To counteract the threat posed by double-strand breaks, cells have evolved multiple, partially overlapping repair mechanisms that differ in accuracy, speed, and template dependence (Cannan & Pederson, 2016; Chapman et al., 2012; Scully et al., 2019). One major pathway is non-homologous end joining (NHEJ), which directly rejoins broken DNA ends with minimal or no requirement for sequence homology and therefore operates rapidly but can introduce small insertions or deletions at the repair junction (Bowater & Doherty, 2006; Chapman et al., 2012; Jackson & Bartek, 2009; Lieber, 2010). A second class of mechanisms, collectively termed homology-directed repair (HDR), relies on the presence of an intact homologous DNA template to restore genetic information with high fidelity (Chapman et al., 2012; Li & Heyer, 2008; San Filippo et al., 2008; Wright et al., 2018). Additional pathways, such as single-strand annealing (SSA) and microhomology-mediated end joining (MMEJ), utilize short homologous sequences flanking the break and are typically associated with deletions or genome rearrangements (Bhargava et al., 2016; McVey & Lee, 2008; Pâques & Haber, 1999). The relative usage of these pathways depends on organism, cell cycle stage, and chromosomal context, reflecting a balance between repair speed and genomic accuracy.

Among these pathways, homology-directed repair (HDR) is the most accurate pathway for the repair of double-strand breaks that restores genetic information by using an intact homologous DNA sequence as a template. This template is typically the sister chromatid in eukaryotes or an undamaged copy of the chromosome in prokaryotes (Kowalczykowski, 2015). By copying sequence information from an unbroken homolog, HDR enables precise repair without the loss or gain of genetic material, making it particularly important for preserving genome integrity following replication-associated damage (Chapman et al., 2012; Heyer et al., 2010).

A defining feature of HDR is its reliance on extensive sequence homology and coordinated DNA processing steps that convert the broken DNA ends into recombinogenic intermediates capable of locating and engaging a homologous template (Kowalczykowski, 2015; San Filippo et al., 2008). As a result, HDR is inherently more complex and slower than direct end-joining

pathways but offers significantly greater accuracy (Jasin & Rothstein, 2013; Mao et al., 2008). Despite these constraints, HDR is evolutionarily conserved and widely utilized, reflecting the fundamental need for error-free repair in contexts where genomic stability is paramount (Kowalczykowski, 2015).

1.1 General Principles of HDR

Homology-directed repair (HDR) proceeds through a series of coordinated molecular steps that transform a broken DNA molecule into a recombination-competent intermediate and ultimately restore chromosome continuity using a homologous template (Fig. 1.2). Although the core logic of HDR is conserved across organisms, the molecular implementation of this pathway differs between prokaryotes and eukaryotes, reflecting differences in genome organization, cell cycle regulation, and chromosomal architecture (Bell & Kowalczykowski, 2016a; Camerini-Otero & Hsieh, 1995). In this section, I will outline the general principles underlying HDR-mediated repair and describe the distinct mechanistic features that characterize HDR in prokaryotic and eukaryotic systems.

1.1.1 HDR in bacteria

In bacteria, double-strand breaks are detected primarily through the appearance of exposed DNA ends, which are highly atypical for intact circular chromosomes. These free DNA ends are rapidly bound by dedicated DNA end-processing complexes, most prominently the RecBCD complex in many Gram-negative bacteria or the functionally analogous AddAB complex in several Gram-positive species (Kowalczykowski et al., 1994; Wigley, 2013; Yeeles & Dillingham, 2007). These complexes serve as the primary sensors and processors of DSBs, coupling break recognition directly to pathway initiation rather than relying on a separate signaling cascade.

Upon binding to a DNA end, RecBCD acts as a highly processive helicase-nuclease complex. It unwinds the DNA duplex while simultaneously degrading both strands in a regulated manner (Cockram et al., 2015; Dillingham & Kowalczykowski, 2008; Taylor & Smith, 2003). Importantly, RecBCD activity is modulated by specific DNA sequence elements known as Chi sites (crossover hotspot instigator sequences) (Dixon & Kowalczykowski, 1993; G. R. Smith, 2012). Encounter with a Chi site alters the enzymatic behavior of RecBCD: nuclease activity on the strand destined for recombination is attenuated, while the production of a 3' single-stranded DNA overhang is promoted (Bell & Kowalczykowski, 2016a; Spies et al., 2003, 2007;

Taylor & Smith, 2003). This regulated processing converts the broken DNA end into a recombination-competent substrate rather than allowing unchecked degradation.

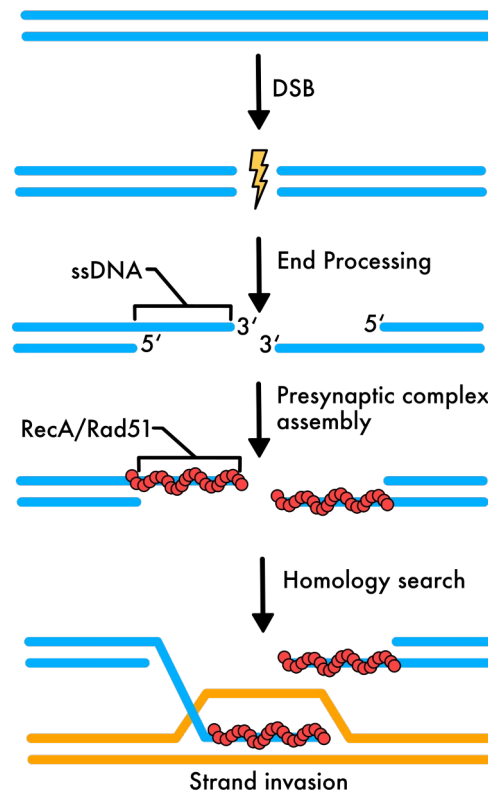


Figure 1.2: Illustration of key steps in HDR mechanism. First, DSB occurs which is followed by the processing of the free ends leading to 3' ssDNA overhangs at the DSB ends. Then ssDNA acts as a substrate for the RecA/Rad51 recombinase, and its polymerization results in presynaptic complex formation. Finally this complex performs the homology search in the genome and after a successful match initiates the strand invasion and repair.

The generation of single-stranded DNA represents a critical commitment point in bacterial HDR. These ssDNA regions are initially coated by single-stranded DNA-binding proteins (SSB), which protect the DNA from secondary structure formation and degradation (Kowalczykowski & Krupp, 1987; Shereda et al., 2008; Wu et al., 2017). Subsequently, SSB is displaced by the recombinase RecA with the assistance of mediator proteins, leading to the formation of a RecA-ssDNA nucleoprotein filament (D. G. Anderson & Kowalczykowski, 1997; Bell & Kowalczykowski, 2016b; Spies et al., 2005; Thresher et al., 1988; Umezumi et al., 1993). This filament is the central catalytic entity of homologous recombination and is responsible for conducting the homology search and promoting strand invasion into an intact homologous DNA molecule (Bell & Kowalczykowski, 2016b; Cox, 1999).

Once a homologous sequence is identified, the RecA filament facilitates strand exchange, pairing the broken DNA end with the complementary strand of the homologous duplex (Bell & Kowalczykowski, 2016b; Cox & Lehman, 1982; Kowalczykowski et al., 1994; Radding, 1982). This generates joint DNA intermediates, such as displacement loops (D-loops), which serve as templates for DNA synthesis (Rao & Radding, 1993; Shibata et al., 1979). DNA polymerases extend the invading strand using the intact chromosome as a guide, thereby restoring lost genetic information. The resulting recombination intermediates are then processed and resolved by branch migration and resolution enzymes, ensuring proper chromosome reconstitution and segregation (Kowalczykowski et al., 1994; J. Liu et al., 1999; West, 1992).

Although homology-directed repair is the dominant pathway for DSB repair in actively growing bacteria, alternative repair options exist. Some bacterial species encode non-homologous end joining (NHEJ) systems, typically involving Ku-like DNA end-binding proteins and a dedicated ligase. These pathways are generally engaged under conditions where homologous templates are unavailable, such as in stationary phase, and are often more error-prone (Bowater & Doherty, 2006; Pitcher et al., 2007; Shuman & Glickman, 2007; Weller et al., 2002). The relative engagement of HDR versus end-joining therefore reflects both environmental conditions and the cell's state, with RecBCD-mediated homologous recombination representing the preferred, high-fidelity solution during normal growth.

1.1.2 HDR in eukaryotes

In eukaryotic cells, double-strand breaks are detected through the rapid recognition of exposed DNA ends by specialized sensor complexes that initiate both repair and checkpoint signaling. One of the earliest responders is the MRN complex (Mre11-Rad50-Nbs1), which binds directly to broken DNA ends and serves as a central hub for damage detection, signaling, and processing (Krogh & Symington, 2004; Lamarche et al., 2010; F. Zhao et al., 2020). Recruitment of MRN facilitates activation of DNA damage checkpoint kinases, coordinating cell-cycle arrest with repair pathway engagement and ensuring that DSBs are not processed inappropriately (Branzei & Foiani, 2008; J.-H. Lee & Paull, 2004; Syed & Tainer, 2018).

Following detection, the fate of a DSB is strongly influenced by cell-cycle stage and chromatin context. In G1 phase, when no sister chromatid is available, DSBs are preferentially repaired by non-homologous end joining (NHEJ), a rapid, template - independent mechanism. In contrast, during S and G2 phases, when an identical sister chromatid is present, breaks are

increasingly routed toward homology-directed repair (HDR). This pathway choice is tightly regulated through control of DNA end resection: suppression of resection favors NHEJ, whereas initiation of resection commits the break to homology-based repair and effectively excludes end joining (Chapman et al., 2012; Shrivastav et al., 2008; Symington & Gautier, 2011).

DNA end resection in eukaryotes proceeds in two stages. Initial short-range resection is catalyzed by the nuclease activity of Mre11 in conjunction with accessory factors, generating limited single-stranded DNA. This is followed by extensive long-range resection mediated by helicase-nuclease complexes and exonucleases, producing long 3' single-stranded DNA overhangs (Mimitou & Symington, 2009; Symington & Gautier, 2011; Zhu et al., 2008). These ssDNA regions are rapidly coated by replication protein A (RPA), which stabilizes the DNA and prevents secondary structure formation (Ruff et al., 2016; Sampathkumar et al., 2024; Sleeth et al., 2007). Subsequent replacement of RPA by the recombinase Rad51, facilitated by mediator proteins, leads to the formation of a Rad51-ssDNA nucleoprotein filament (Sleeth et al., 2007; Sugiyama & Kowalczykowski, 2002; Sung, 1997).

The Rad51 filament constitutes the core catalytic unit of eukaryotic homologous recombination (Baumann et al., 1996; Sung, 1994). It conducts a genome-wide homology search to identify a matching sequence on the sister chromatid and promotes strand invasion into the intact duplex DNA (Dumont et al., 2024; S. Liu et al., 2023; Pâques & Haber, 1999; Qi et al., 2015; Sung & Robberson, 1995). This process generates joint DNA intermediates, such as displacement loops (D-loops), which serve as templates for repair DNA synthesis. Extension of the invading strand restores missing genetic information, after which recombination intermediates are resolved to re-establish chromosome integrity (Heyer et al., 2010; McIlwraith et al., 2005; McIlwraith & West, 2008).

In addition to Rad51, eukaryotes encode a second RecA-family recombinase, Dmc1, which plays a specialized role in homologous recombination during meiosis (Bishop et al., 1992; Hinch et al., 2020). Unlike Rad51, which functions broadly in somatic cells throughout the cell cycle, Dmc1 is expressed and activated specifically during meiotic prophase, where it facilitates recombination between homologous chromosomes rather than sister chromatids (Neale & Keeney, 2006; Schwacha & Kleckner, 1997). This distinction is critical for the generation of crossovers that ensure accurate homolog segregation and promote genetic diversity. Although Rad51 and Dmc1 share structural and mechanistic similarities, their activities are differentially regulated, and their coordinated action during meiosis biases recombination toward inter-homolog interactions (Brown & Bishop, 2015; Steinfeld et al.,

2019). The existence of Dmc1 thus highlights the functional diversification of recombinases in eukaryotes and underscores how homologous recombination pathways are adapted to distinct biological contexts.

In parallel, non-homologous end joining operates as a major alternative repair pathway, particularly in G1 and in non-dividing cells (van Gent et al., 2001). NHEJ is initiated by rapid binding of Ku proteins to DNA ends, which protect the break and recruit additional factors that bridge and align the DNA termini (Lieber, 2010; Mari et al., 2006; Walker et al., 2001). Limited end processing may occur to render the ends compatible for ligation, after which a dedicated ligase complex rejoins the DNA ends (Critchlow & Jackson, 1998). Although efficient and fast, NHEJ often introduces small insertions or deletions at the repair junction, reflecting its inherently error-prone nature (Chang et al., 2017). Together with alternative end-joining pathways that utilize short microhomologies, NHEJ provides a critical but less accurate means of DSB repair when homologous templates are unavailable.

Although homology-directed repair is evolutionarily conserved, its molecular implementation differs markedly between prokaryotes and eukaryotes, reflecting fundamental differences in genome organization, cellular architecture, and regulatory complexity. In bacteria, HDR is tightly coupled to chromosome replication and operates within a compact, unsegregated nucleoid, where homologous templates are often nearby in space and repair is initiated directly by DNA end-processing complexes such as RecBCD (Kuzminov, 1999). In contrast, eukaryotic HDR takes place within a membrane-bound nucleus containing multiple linear chromosomes packaged into chromatin, and repair pathway choice is strongly regulated by cell-cycle stage and checkpoint signaling (Heyer et al., 2010). These differences influence how DSBs are detected, processed, and committed to repair, as well as how recombination intermediates are resolved.

Despite these organism-specific distinctions, a central mechanistic challenge is shared across all domains of life: the accurate and timely search for a homologous DNA sequence. In both prokaryotes and eukaryotes, recombinase-coated single-stranded DNA must locate a matching sequence within a vast excess of non-homologous DNA (Bell & Kowalczykowski, 2016b; Forget & Kowalczykowski, 2012; Hu & Crickard, 2024; Prentiss et al., 2015; Renkawitz et al., 2014; Savir & Tlusty, 2010). This task is nontrivial given the size of genomes, the crowded intracellular environment, and the need to avoid erroneous interactions that could lead to genome rearrangements. The fact that cells routinely complete homology search on biologically relevant timescales suggests the existence of highly optimized strategies that go beyond simple diffusion-limited encounters (Barzel & Kupiec, 2008).

Thus, while the regulatory frameworks and molecular players of HDR differ between bacteria and eukaryotes, the physical problem of homology search is fundamentally universal. Understanding how cells overcome this challenge is therefore key to explaining the robustness and efficiency of homologous recombination across diverse biological systems. This shared constraint provides a unifying lens through which the dynamics of recombinase filaments and chromosome organization can be examined.

1.2 Homology search as a spatiotemporal optimization problem

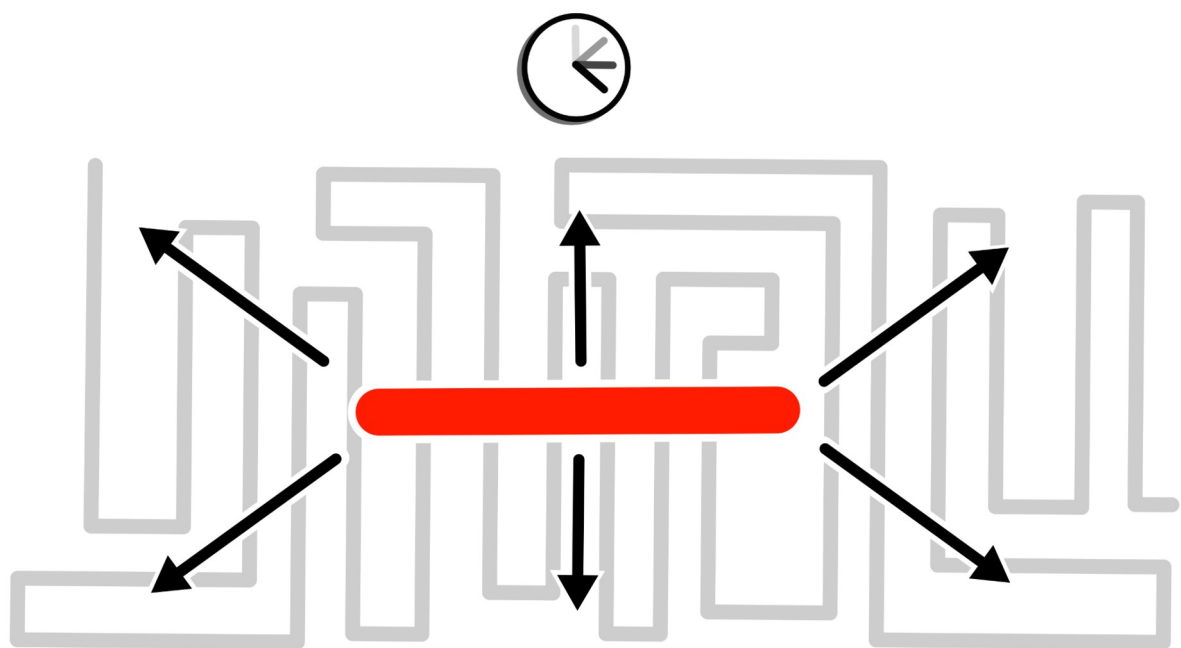


Figure 1.3: Illustration of homology search by recombinase filament (red) as a spatial and temporal optimization problem against large genomic content (grey) and fast repair before the DSB site gets irreversibly damaged, respectively.

At the heart of homology-directed repair lies the challenge of locating a matching DNA sequence within a crowded and dynamically organized genome. This task is inherently a spatiotemporal optimization problem, requiring cells to balance search speed, accuracy, and resource expenditure, as illustrated in Fig. 1.3. A recombinase-bound single-stranded DNA substrate must identify a homologous sequence among millions to billions of base pairs while avoiding spurious interactions with non-homologous regions. Simple scanning and diffusion-based search mechanisms would be prohibitively slow at cellular length scales, particularly given the dense packing of chromosomes and the limited time window imposed by ongoing replication, transcription, and cell division.

This limitation is underscored by measurements of bacterial chromosomal dynamics. Chromosomal loci in *E. coli* move subdiffusively rather than by rapid free diffusion with extrapolated timescales of roughly years to passively explore distances of a few micrometers, comparable to the length of a bacterial cell (Weber et al., 2010). Thus, both spatial diffusion of genomic loci and progressive sequence-by-sequence interrogation are far too slow to explain homology search on biologically relevant timescales without additional organizational or facilitated-search mechanisms.

To illustrate this challenge quantitatively, consider a simplified estimate for homology search in *E. coli*. Progressive DNA sequence scanning by a RecA-ssDNA filament would require sampling a very large number of potential genomic positions before locating the correct repair template. The *E. coli* K-12 genome contains 4,639,221 base pairs (Blattner et al., 1997). For a RecA filament carrying approximately 1 kb of ssDNA, the number of possible 1-kb alignment windows per chromosome is

$$W = L - 1000 + 1 \approx 4.64 \times 10^6$$

In a dividing cell containing two chromosome equivalents, but with only one intact homologous target site on the sister chromosome (the corresponding region on the broken chromosome being disrupted by the DSB), the effective search space becomes

$$N \approx 2W \approx 9.28 \times 10^6$$

candidate windows with a single correct target.

Even assuming an extremely optimistic interrogation time of ~30 ms per candidate site, based on single-molecule measurements of rapid RecA-mediated homology testing upon dsDNA encounter (Ragunathan et al., 2012), the expected search time would be

$$(N/2) \times 0.03s \approx 1.4 \times 10^5s$$

or approximately 39 hours. Thus, a progressive sequence scanning model predicts homology search times on the order of days.

In contrast, experimental measurements indicate that homology search in bacteria occurs on the order of tens of minutes in-vivo (Chimthanawala et al., 2022; Wiktor et al., 2021). Similar scaling arguments applied to eukaryotic genomes lead to even more dramatic discrepancies: for *Saccharomyces cerevisiae* (~12.1 Mb), a naive scanning-based estimate predicts search times on the order of ~2 days, while for the human genome (~3.2 Gb) the predicted timescale extends to 1.5-3 years. However, experimental observations suggest that homology search

occurs much more rapidly, taking approximately 3-4 hours in yeast (S. Liu et al., 2023) and 10-16 hours in human cells (Friskes et al., 2025), several orders of magnitude faster than diffusion-limited predictions.

Efficient homology search therefore demands strategies that optimize both spatial sampling and temporal progression. Spatially, the search machinery must effectively explore large chromosomal volumes without becoming trapped in local neighborhoods or constrained by topological barriers. Temporally, interactions must be sufficiently long-lived to allow sequence interrogation, yet transient enough to permit rapid disengagement from incorrect targets. These competing requirements imply that homology search is not a random process, but rather one shaped by filament dynamics, chromosome organization, and active cellular processes that bias encounters toward productive outcomes.

Framing homology search in this way highlights a fundamental tension shared across organisms: how to achieve rapid, genome-wide sampling while maintaining high specificity. This perspective naturally shifts the focus from purely biochemical descriptions of recombination toward the physical principles governing macromolecular motion and DNA organization. Understanding how cells resolve this optimization problem is essential for explaining the robustness and efficiency of homologous recombination under physiological conditions.

1.3 Intuitive solution for homology search in bacteria

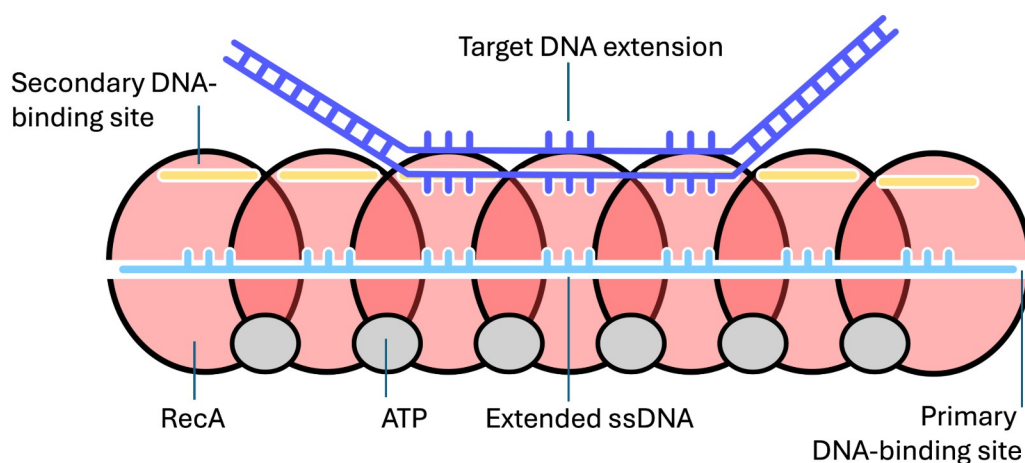


Figure 1.4: Illustration of structural features that facilitate RecA filament mediated homology detection and search. This figure is adapted from (Renkawitz et al., 2014)

As discussed above, homology search in bacteria is mediated by the RecA nucleoprotein filament, whose structural and biochemical properties are critical for efficient search (Bell & Kowalczykowski, 2016b). Several structural features of the filament facilitate homology recognition, such as extension of the bound single-stranded DNA (ssDNA), its organization into repeating triplet units that allow multiple segments of the filament to probe homology in parallel, and its high stiffness, which increases the effective reach of the filament within the nucleoid (Bell & Kowalczykowski, 2016b; Lesterlin et al., 2014; Prentiss et al., 2015; Ragunathan et al., 2012; van Loenhout et al., 2009). Below I review these and other key structural and mechanistic features of RecA filament that are considered to facilitate homology search in bacteria, few of which are highlighted in Fig. 1.4.

RecA assembles on ssDNA to form a helical nucleoprotein filament in which each RecA monomer covers approximately three nucleotides, giving rise to the triplet organization that underlies parallel homology probing. In the ATP-bound state, the filament has a pitch of ~9-10 nm with roughly six monomers per turn and a diameter of ~10 nm (Chen et al., 2008; Di Capua et al., 1982; Egelman & Stasiak, 1986; Heuser & Griffith, 1989; Story et al., 1992). Within this structure, the ssDNA is extended to approximately 1.5 times the length of B-form duplex DNA of the same sequence, a property that facilitates alignment with potential homologous regions during strand interrogation (Chen et al., 2008; Dunn et al., 1982). The filament is also mechanically stiff, with a persistence length of ~1 μm compared to ~50 nm for naked dsDNA (Hagerman, 1988; Hegner et al., 1999; van Loenhout et al., 2009). This increased rigidity allows the filament associated with a double-strand break to extend into surrounding regions of the nucleoid and sample DNA sequences beyond the immediate vicinity of the break. In contrast, ADP-bound filaments adopt a more compact and flexible conformation, which may facilitate filament remodeling during the search process (J. Y. Lee et al., 2016; B. Zhao et al., 2017).

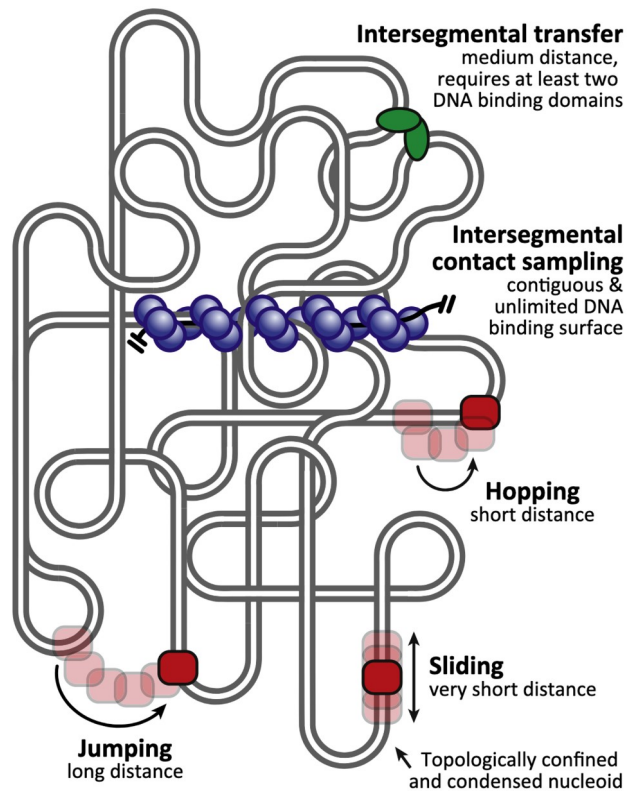


Figure 1.5: Illustration of different modes by which DNA binding proteins find their targets. This figure is adapted from (Bell & Kowalczykowski, 2016b).

The RecA filament interrogates duplex DNA through transient interactions with potential target sequences. However, stable pairing requires a minimal stretch of homology, typically around eight nucleotides, which serves as a threshold for productive strand exchange (Qi et al., 2015; Ragunathan et al., 2012). Early theoretical models proposed several diffusion-based mechanisms by which DNA-binding proteins locate specific DNA sequences, including sliding, hopping, jumping, and inter-segmental transfer, illustrated in Fig. 1.5 (Bell & Kowalczykowski, 2016b; Von Hippel & Berg, 1989). Sliding refers to one-dimensional diffusion along the DNA while maintaining continuous contact, enabling local sequence scanning but limiting long-range exploration. Hopping involves brief dissociation and reassociation events over short distances, allowing proteins to bypass obstacles and sample nearby regions more rapidly than pure sliding. Jumping consists of longer-range dissociation into solution followed by rebinding at distant sites, facilitating rapid exploration of the genome but without continuous scanning. Inter-segmental transfer occurs when a protein transfers directly between two nearby DNA segments in three-dimensional space, enabling efficient sampling of distant genomic regions without full dissociation.

Initial experimental studies of RecA-mediated homology search suggested that target recognition occurs primarily through three-dimensional collisions between the RecA filament

and duplex DNA rather than through extensive one-dimensional sliding along DNA (Adzuma, 1998). Subsequent experiments have revealed that inter-segmental transfer plays an important role in homology search, with the three-dimensional conformation of dsDNA and the length of the RecA filament influencing search efficiency (Bell & Kowalczykowski, 2016b; Forget & Kowalczykowski, 2012). Single-molecule studies have also shown that RecA filaments frequently form transient interactions with heterologous DNA loci (Forget & Kowalczykowski, 2012; Ragunathan et al., 2012). These interactions can generate looped intermediates connecting distant regions of DNA or allow short segments of duplex DNA to slide along the filament over distances of roughly 60-300 bp before dissociation (Ragunathan et al., 2012). Taken together, these observations have led to the proposal of an intersegmental contact sampling mechanism, in which a single RecA filament forms multiple transient interactions with a coiled dsDNA molecule, enabling simultaneous probing of different genomic regions and thereby accelerating homology search.

Most of the mechanistic insights into RecA-mediated homology search were initially derived from in-vitro biochemical and single-molecule experiments, leaving open the question of how RecA filaments behave inside living cells. Early live-cell imaging studies demonstrated that RecA forms extended nucleoprotein structures following DNA damage. For example, fluorescence microscopy experiments in *Escherichia coli* revealed the formation of RecA bundles or extended foci spanning much of the cell length during double-strand break repair (Lesterlin et al., 2014; Renzette et al., 2005). These structures occupy the region between the nucleoid and the cell membrane and consist of a relatively immobile central core with thinner dynamic extensions (Lesterlin et al., 2014). Similar observations have been reported in other bacterial species. In *Bacillus subtilis*, RecA was shown to assemble into elongated filamentous structures that dynamically redistribute across the nucleoid during the homology search process (Kidane & Graumann, 2005). These studies established that RecA-mediated repair involves the formation of large-scale nucleoprotein assemblies that can extend across substantial portions of the bacterial cell.

Subsequent imaging studies with improved temporal and spatial resolution have further refined this view of RecA organization in-vivo. More recent work using single-molecule and super-resolution approaches has revealed that RecA often forms thinner and highly dynamic filaments rather than large static bundles (Ghodke et al., 2019; Wiktor et al., 2021). In these systems, RecA filaments extend across the whole cell. Based on these observations, it has been proposed that this organization enables homology search through a reduced-dimensionality mechanism that enables rapid genome-wide interrogation. Their theoretical estimates suggest that when RecA filament is extended through the whole cell, donor DNA

diffusion takes only 5-10 min to find the correct sequence on the filament. Consistent with this model, in *E. coli* cells, after DSB induction, the homology search was completed within ~10 minutes (Wiktor et al., 2021). Together, these observations suggest that RecA-mediated homology search in-vivo involves highly dynamic nucleoprotein filaments capable of scanning large genomic regions efficiently.

Recent live-cell imaging studies have shown that RecA filaments formed during double-strand break repair can undergo directed pole-to-pole translocation across the bacterial cell. In *Caulobacter crescentus*, this movement allows the RecA-ssDNA filament to explore different regions of the chromosome during homology search. This translocation is mediated by the SMC-like protein RecN, which associates with RecA during repair (Chimthanawala et al., 2022). Loss of RecN abolishes filament movement and leads to severe defects in double-strand break repair, indicating that RecN plays a key role in regulating RecA filament dynamics during homology search.

1.4 RecN is conserved in all bacteria

RecN is a bacterial SMC (structural maintenance of chromosome) complex that participates in DSB repair (Funayama et al., 1999; Picksley et al., 1984; Sanchez et al., 2008). Although it shares the hallmark ATPase architecture of SMC proteins, RecN is structurally simpler than canonical SMC complexes involved in chromosome condensation or segregation (Fig. 1.6). Structural work on *Deinococcus radiodurans* RecN showed that the protein contains conserved N- and C-terminal ATPase head domains connected by coiled-coil regions, consistent with an SMC-like organization adapted for DNA repair rather than global chromosome maintenance. This streamlined architecture is thought to support rapid engagement with broken DNA and dynamic remodeling during homologous recombination (Pellegrino et al., 2012).

In-vitro experiments have begun to clarify how RecN interfaces with both DNA and RecA. RecN from *D. radiodurans*, in-vitro, showed enhanced intermolecular DNA interactions (Reyes et al., 2010). Purified *E. coli* RecN loads topologically onto both ssDNA and dsDNA, with a preference for ssDNA, and can capture a second DNA molecule in trans, supporting a DNA-tethering or synapsis-promoting role during repair (Keyamura & Hishida, 2019). Earlier work also showed that RecA recruits RecN to DSB-associated DNA substrates and that RecN contributes to the formation of recombination-competent repair assemblies (Keyamura et al., 2013). In addition, biochemical analysis demonstrated that RecN stimulates the DNA strand invasion step of RecA-mediated homologous recombination, enhancing D-loop formation and

strand exchange in-vitro (Uranga et al., 2017). Together, these results support a model in which RecN acts upstream of productive donor engagement by stabilizing DNA contacts and promoting interactions between RecA-coated ssDNA and homologous duplex DNA.

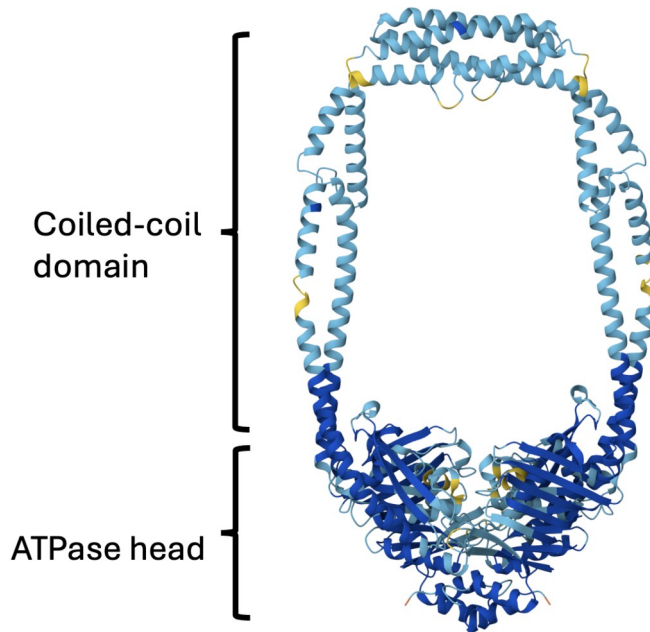


Figure 1.6: AlphaFold3 (Abramson et al., 2024) predicted structure of RecN homodimer from *E. coli*.

In-vivo, RecN has emerged as a key organizer of bacterial DSB repair, with roles spanning early damage response to chromosome-scale organization. Early studies in *E. coli* showed a central role of RecN in drug induced cytotoxicity (Kosa et al., 2004), as well as recombinational repair (Meddows et al., 2005). Further studies suggested that RecN forms nucleoid associated foci (Kidane et al., 2004; Kidane & Graumann, 2005; Nagashima et al., 2006), and its interaction with RecA filament is crucial for localization (Keyamura et al., 2013; McLean et al., 2021). More recently, RecN, together with RecA, has been shown to drive a large-scale DNA supercompaction response following DNA damage, indicating that these proteins can actively reorganize the chromosome to facilitate repair (Vickridge et al., 2017; Vikedal et al., 2025). However the reorganization was limited to the regions proximal to the DSB site and didn't lead to whole chromosome alignment. Extending these observations, live-cell imaging in *Caulobacter crescentus* revealed that RecN regulates RecA filament dynamics during homology search by enabling directed pole-to-pole translocation and efficient chromosome-wide exploration (Chimthanawala et al., 2022; Noda et al., 2023). Together, these findings establish RecN as a conserved regulator that couples recombination filament activity with global chromosome organization during DNA repair.

Despite significant progress in understanding the structural, biochemical, and cellular roles of RecN, the mechanisms by which it drives large-scale RecA filament dynamics remain poorly understood. In particular, while recent studies have demonstrated that RecN enables directed pole-to-pole translocation of RecA filaments during homology search, the underlying molecular basis of this motion remains unclear. Furthermore, most of these dynamic insights have been obtained in a limited number of model organisms, and it is not yet known to what extent RecN-mediated regulation of RecA filament motion is conserved across different bacterial species. Addressing these questions is essential for developing a unified understanding of how homologous recombination is coordinated across spatial scales, from local DNA processing to chromosome-wide search dynamics.

1.5 Aims of the thesis

As discussed above, recent experiments have shown that RecN drives pole-to-pole translocations of RecA filament during homology search, and that loss of RecN severely impairs double-strand break repair. Despite its conservation across bacteria and its essential role in recombination, the mechanistic basis of RecN function remains poorly understood. In particular, it is unclear how RecN interacts with RecA filaments and DNA to generate coordinated, cell-scale motion during homology search. Addressing this question is challenging, in part due to the lack of quantitative frameworks that can link molecular interactions to emergent large-scale dynamics in the cellular environment. Therefore, there is a need for approaches that enable the formulation and testing of mechanistic models capable of explaining how RecN regulates RecA filament motion during DNA repair.

The key aims of my doctoral thesis are:

1. Formulate a hypothesis for the mechanistic role of the RecN in homology search in bacteria, specifically *C. crescentus*.
2. Build a coarse-grained molecular dynamics polymer simulation model to investigate the proposed hypothesis.
3. Determine the key aspects for the efficient homology search during DSB repair.
4. Test the feasibility of the proposed model in other bacteria.

To address this gap, first, I hypothesize that RecN can work as, RecA filament associated, simple DNA motor, like other SMCs. While being attached to RecA, RecN can simultaneously interact with DNA coupled with structural changes that can pull the DNA in one direction and push the filament in another. To test this hypothesis, I constructed a polymer-based simulation model of the bacterial chromosome, calibrated to reproduce DNA dynamics observed in-vivo. Within this framework, the RecA filament was modeled as a stiff polymer chain, while RecN was represented as interacting particles associated with the filament. Using this model, I tested a motor-like mechanism for RecN-driven filament motion and found that it enables efficient homology search and recognition at the cellular scale. The simulations further revealed that key factors governing this motion include the mechanical stiffness of the RecA filament, the spatial organization of DNA along the filament, the localization patterns of RecN, and its interaction with surrounding DNA. Finally, the generality of this mechanism was examined by extending the model to different cellular geometries, including an *E. coli*-like system and spherical confinement, where RecN-mediated dynamics remained essential for efficient repair.

2. Results

2.1 Motor-like motion of RecN generates cell pole-to-pole RecA filament translocations in-silico

To understand how RecN drives large-scale RecA filament motion during homology search, as observed experimentally, I developed a coarse grained polymer simulation framework that captures the organization of the bacterial cell. A brief description of the simulation model is provided here, for further details see Methods section 4.1. The model explicitly incorporates chromosomal DNA, the RecA filament, and RecN, enabling mechanistic investigation of the homology search process. Whole bacteria is modeled as a cylindrical cell with dimensions of a typical *C. crescentus* cell (5 μm length and 0.6 μm diameter), as illustrated in Fig. 2.1. To model the replicating cell I have enclosed 2 sister chromosomes, and their initial configuration is generated as a random walk in their respective cell half. DNA in those chromosomes is modeled as a freely jointed chain with excluded volume, and monomers in each chain are connected via simple harmonic bonds (Fig. 2.1, left inset).

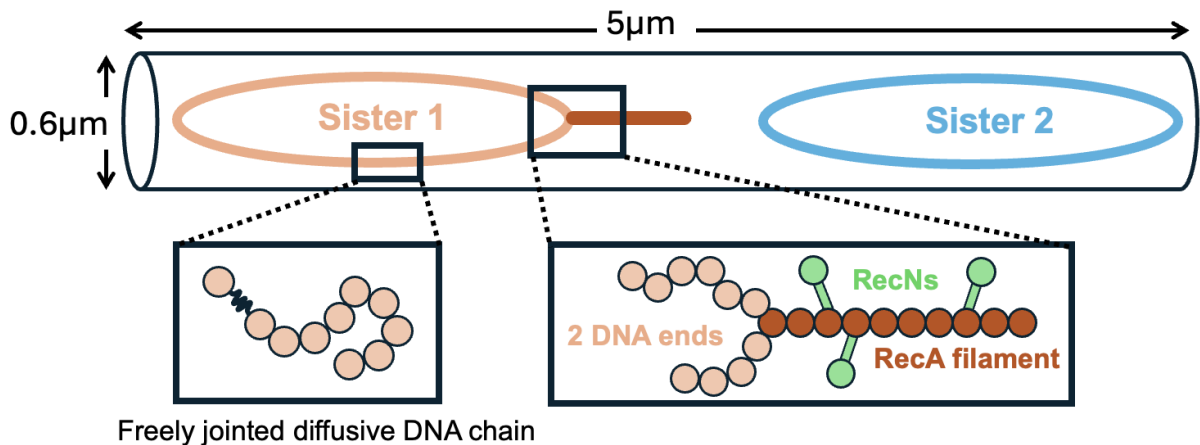


Figure 2.1: Schematic of the simulated bacterial cell and its different components.

The dynamics of the DNA in the simulation are calibrated to reproduce the diffusive behavior of chromosomal DNA loci observed in-vivo (for details see Methods section 4.1.2). Specifically, model parameters are tuned such that the mean-squared displacement (MSD) of simulated DNA segments matches experimentally measured diffusion profiles in bacterial cells (Weber et al., 2010). This calibration establishes a direct mapping between simulation time units and physical time, allowing the temporal evolution of the system to be quantitatively compared with experimental observations. As a result, the simulated dynamics not only

capture the correct spatial fluctuations of DNA but also operate on a realistic timescale, ensuring that processes such as RecN-driven filament reorganization can be interpreted in the context of biologically relevant time frames.

The RecA filament is modeled as a stiff polymer chain with enhanced rigidity, reflecting its experimentally observed persistence length and extended conformation. The stiffness of the filament is controlled by a simple harmonic angle constraint, which maintains the persistence length of $\sim 1 \mu\text{m}$, as recorded in multiple experiments (Hegner et al., 1999; van Loenhout et al., 2009). Length of the filament is kept fixed at $\sim 1 \mu\text{m}$, which was the experimentally observed average RecA filament length in *C. crescentus* (Chimthanawala et al., 2022). In our model, the RecA filament is assumed to keep the two ends of the double-strand break together, effectively forming a single connected unit (Fig. 2.1, right inset), as suggested by experiments (Dumont et al., 2024). RecN is modeled as discrete particles associated with the RecA filament (Fig. 2.1, right inset), and it can reversibly bind to DNA, with binding events accompanied by changes in its conformation. In our base model there are 20 such motors, randomly placed on the filament. Further details of the RecN motor are given in Methods section 4.1.5-7.

Having defined the structural components of the model, the mechanistic rules governing RecN activity are next specified based on experimental observations. RecN has been reported to interact simultaneously with double-stranded DNA and the RecA filament, with its ATPase activity modulated by these interactions (Keyamura et al., 2013; Uranga et al., 2017). These observations suggest that RecN does not act as a passive structural factor, but rather actively couples ATP hydrolysis to DNA-dependent interactions. Given that RecN belongs to the SMC family of proteins, which are known to function as DNA-associated motors, I propose that RecN may similarly operate as a DNA motor. However, unlike canonical SMC complexes that form stable, processive assemblies through kleisin-mediated structures, RecN lacks such known architectural components. I therefore hypothesize that RecN functions as a transient DNA motor, binding to DNA and generating short-lived, ATP-driven motion before dissociating. Altogether, this leads to a model in which RecN acts as a RecA filament-associated transient DNA motor capable of driving large-scale filament translocation.

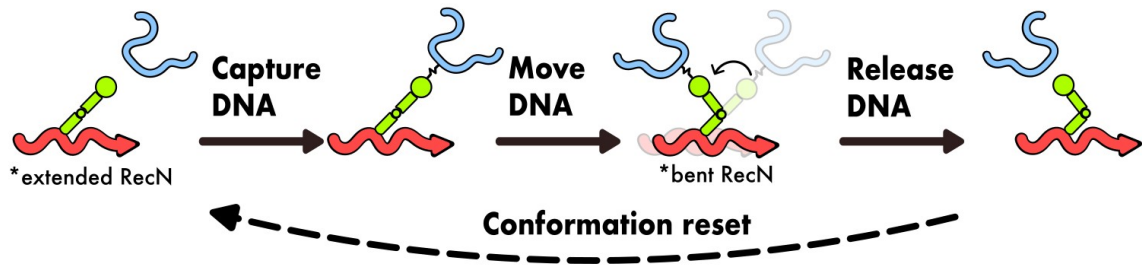


Figure 2.2: Schematic of proposed RecN motor mechanism.

To model the RecN as a simple DNA motor, first, it is assumed that RecN has a specific binding site on RecA monomers. Second, RecN is oriented in such a manner that in the DNA unbound phase RecN faces towards the tip of the RecA filament, in an extended conformation, as illustrated in Fig. 2.2 (for further explanation see Methods section 4.1.6). Now, the motor works in primarily 4 steps, which are as follows:

1. In the extended conformation, RecN has affinity for DNA segments, and thus may lead to the capture of a nearby DNA segment.
2. Once there is a DNA segment within the capture radii of the motor, it can stably bind to that segment.
3. DNA binding then triggers the conformational change to the bend configuration where RecN now orients towards the rear of the filament, as shown in the third step in Fig. 2.2.
4. Following this, the bend configuration leads to DNA unbinding/release and RecN orientation resets to the extended state.

In this cycle, the third step constitutes the key force-generating step, in which RecN pulls the DNA toward the tail of the filament. This results in a forward displacement of the filament toward the tip. We hypothesize that, when multiple such motors are bound to the RecA filament, their combined activity generates a cumulative directional motion of the filament, as observed in-vivo.

To test whether RecN's proposed motor mechanism explains large-scale filament dynamics, I implemented it in the polymer simulation framework described above. RecN activity is modeled via state-dependent interactions governing DNA binding and conformational changes. The detailed implementation of these rules and parameters is described in the Methods section 4.1.6-7. With the model fully specified, the next step is to examine whether the proposed mechanism can reproduce the experimentally observed filament motion.

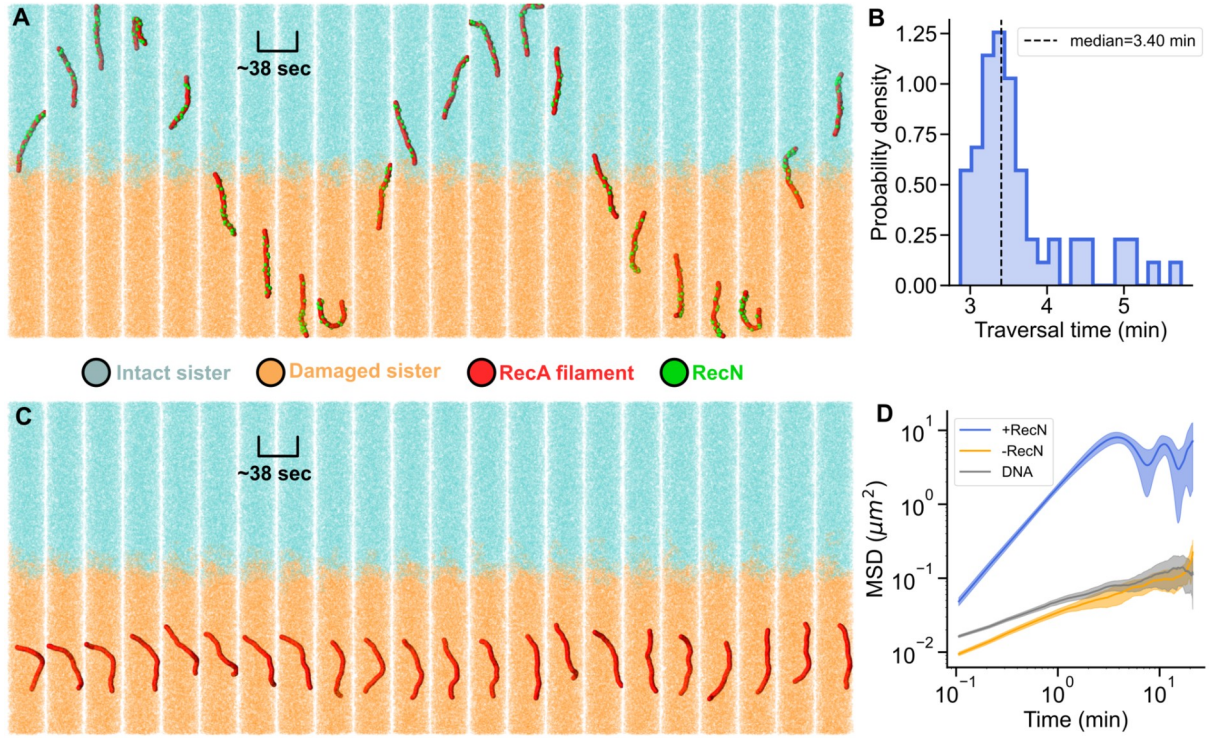


Figure 2.3: RecA filament cell pole-to-pole traversals assisted by proposed RecN motor action. (A) Simulation trajectory showing RecA filament (red) associated with multiple RecNs (green dots) performing cell pole to pole traversals across damaged (orange) and intact (cyan) chromosome. **(B)** Distribution of the complete traversal time (from one cell pole to another), with a median of 3.4 minutes. **(C)** Simulation trajectory showing absence of traversals when RecN is not present on the RecA filament. **(D)** Mean squared displacement (MSD) of RecA filament with (blue) and without (orange) RecN, and DNA (gray) as a reference.

The simulations indicate that the proposed mechanism is sufficient to generate robust pole-to-pole translocation of the RecA filament within the cell (Fig. 2.3A). For complete cell traversals the median time is ~3.4 min (Fig. 2.3B) which is comparable to experimentally observed traversal times (Chimthanawala et al., 2022). Therefore our model not only captures the qualitative feature of the filament motion but also the experimentally observed traversal times. In contrast, in absence of RecN, the filament remains largely immobile and lacks any long range movement (Fig. 2.3C). Furthermore, mean squared displacement (MSD) indicates that its motion is comparable to DNA in our simulation (Fig. 2.3D). So having just the stiff filament is not sufficient for long range filament motion.

Having established that the model reproduces RecA filament cell pole-to-pole traversals, I next assessed the homology search times and efficiency predicted by model. Search efficiency is defined as the fraction of cells finishing homology search within 20 min, corresponding approximately to the average search time observed experimentally (Chimthanawala et al., 2022). To simulate the filament-donor pairing event I have introduced

a short range attraction between corresponding filament-donor monomers. The interaction is sequence specific, i.e. only matching pairs experience attraction, thereby mimicking homology recognition (Fig. 2.4, for further details see Methods section 4.1.8).

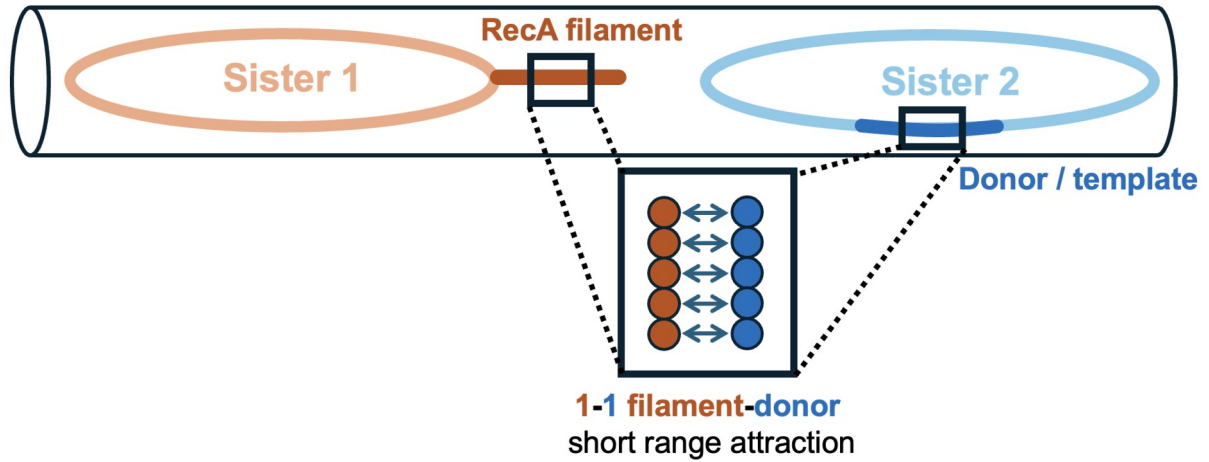


Figure 2.4: Schematic for RecA filament - DNA donor pairing in simulation via monomer pairs specific short range attraction.

As a result of introducing filament-donor attraction, in most of the simulations, RecA filament locates the homologous donor rapidly (Fig. 2.5A). The corresponding homolog search efficiency from these simulations is ~95%, indicating that in majority of the cells RecA filament finds the donor on the other sister chromosome within 20 min (Fig. 2.5B). Furthermore, 50% of cells completed the search within 5 min. In contrast, in the absence of RecN, no successful homology search events were observed in any simulation within the 20 min observation window. The search times predicted by the simulations are therefore substantially shorter than the experimentally observed search times of 20-30 min reported for *C. crescentus* in-vivo (Chimthanawala et al., 2022). Thus, the simulations are best interpreted as providing a lower bound for the timescale of RecN-assisted homology search in cylindrically shaped bacteria.

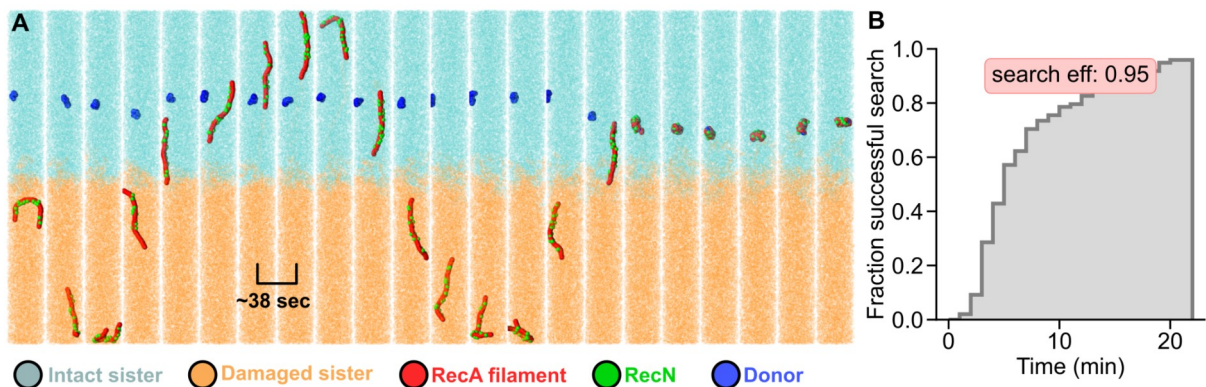


Figure 2.5: Homology search performed by RecA filament. (A) Simulation trajectory showing one example of homology search performed by RecA filament (red), which took multiple traversals to find the donor (dark blue). **(B)** Search timings, measured as the time taken by the filament to find the donor, plotted as a cumulative distribution, search efficiency is calculated as the fraction of cells finishing search within a 20 min time window.

2.2 Force generated by the motors on the filament

While these results demonstrate that RecN-driven filament motion is sufficient to achieve homology search, it is crucial to determine whether the underlying motor dynamics are biophysically realistic. In particular, I examined whether RecN operates within a physiologically reasonable force regime. Cytoskeletal motors such as myosin, dynein, and kinesin typically generate stall forces in the range of 1-10 pN (Roberts et al., 2013; Svoboda & Block, 1994; Sweeney & Holzbaur, 2018), which is a measure of resisting force at which molecular motors stop moving forward. Whereas SMC-family proteins are considerably weaker, with forces on the order of 0.1-1 pN (Ganji et al., 2018; Golfier et al., 2020; Kim et al., 2019; Pobegalov et al., 2023). Although RecN belongs to the SMC family of relatively weak motors, it drives large-scale filament motion in our simulations. I therefore quantified the effective force generated by RecN in our simulation to assess whether such behavior is consistent with biologically realistic force scales.

The average force generated by RecN, in our model, can be estimated by dividing the energy difference between the extended and bent conformations by the distance travelled. This gives an estimated force of ~ 2.4 pN during the force-generating phase of the conformational cycle (see Methods section 4.1.7). Because this phase accounts for approximately 25% of the full cycle duration, the cycle-averaged force is ~ 0.6 pN, which is comparable to the force generated by other molecular motors. However, for filament translocations, the relevant quantity is the total force exerted collectively by RecN motors on the filament, which cannot be accurately estimated using theory alone. Therefore, to estimate the collective stall force generated by RecNs bound to the filament, I performed simulations of a freely moving filament and applied an opposing force at its trailing end, as illustrated in Fig. 2.6A. By progressively increasing this counteracting force, I determined the minimum force required to halt the directed motion of the filament. The force vs velocity plot showed that an opposing force of ~ 1 pN was sufficient to stall the filament (Fig. 2.6B, for exact calculations see Methods section 4.4). This transition is also visible in the trajectory snapshots (Fig. 2.6C-E). Below 1 pN, the filament undergoes sustained pole-to-pole traversals, whereas near 1 pN the motion becomes intermittent and step-like. Above 1 pN, directed motion is completely suppressed and the filament remains stalled near one end of the cell.

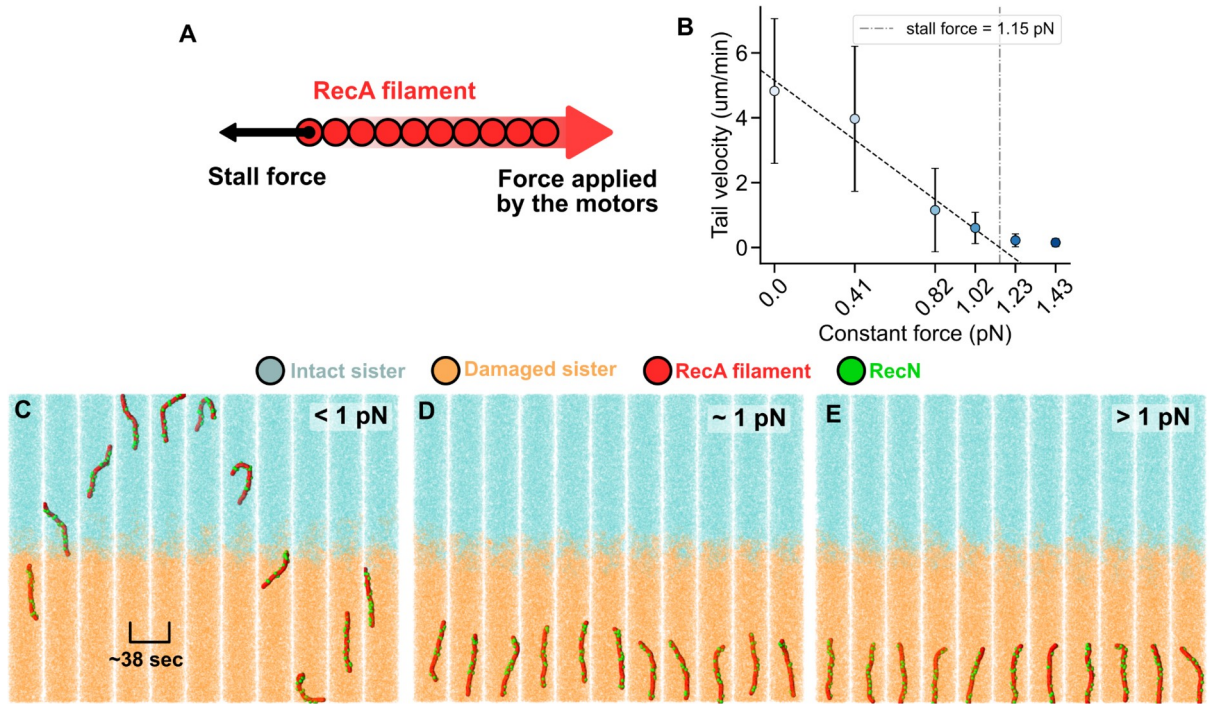


Figure 2.6: Collective forward pushing force generated by the RecN motors on the RecA filament. (A) Illustration of the method used to determine the stall force required to stop the filament motion. RecN motors push the filament in tip facing direction and stall force is applied to the last particle in the filament (i.e. tail). (B) Stall force measurement, constant stall force applied at the filament tail vs the forward velocity of the tail. Only the first 3 points were taken into account to determine the stall force as at ~1 pN filament forward motion stopped. (C-E) Simulation trajectory showing filament motion when <1 pN, ~1 pN, and >1 pN force applied to the tail of the filament, respectively. For details see methods.

A collective stall force of approximately 1 pN for a filament containing 20 RecN motors corresponds to an effective contribution of approximately 0.05 pN per RecN, assuming that all motors contribute equally to the net propulsive force. This value is substantially lower than the local force estimated from the idealized conformational change of a single RecN motor. The difference indicates that only a fraction of the local motor force is transmitted into productive filament-scale motion. Thus, the stall-force measurement should be interpreted as an effective force output of the collective motor-filament system rather than the intrinsic force produced by an isolated RecN molecule.

2.3 RecN motors counter the drag imposed by the trailing DNA behind the RecA filament

While RecN motors drive the translocation of the RecA filament, the filament remains attached to DNA at the double-strand break site. As a result, its motion is expected to be influenced not

only by motor activity but also by mechanical resistance from the attached DNA. In particular, the trailing DNA may impose a drag force that opposes filament movement. To investigate this possibility, I examined how different modes of DNA attachment affect filament dynamics (illustrated in Fig. 2.7A). I first compared the base simulations, in which two DNA ends are attached to the filament, with a free filament that is detached from the chromosome. In the absence of DNA attachment, the filament speed increases substantially, by up to ~4-fold (Fig. 2.7B), suggesting that the trailing DNA imposes a significant drag force on filament motion. Next, I compared the three configurations shown in Fig. 2.7A and found that filament speed increases as the number of attached DNA chains decreases (Fig. 2.7B), indicating that trailing DNA restricts filament motion.

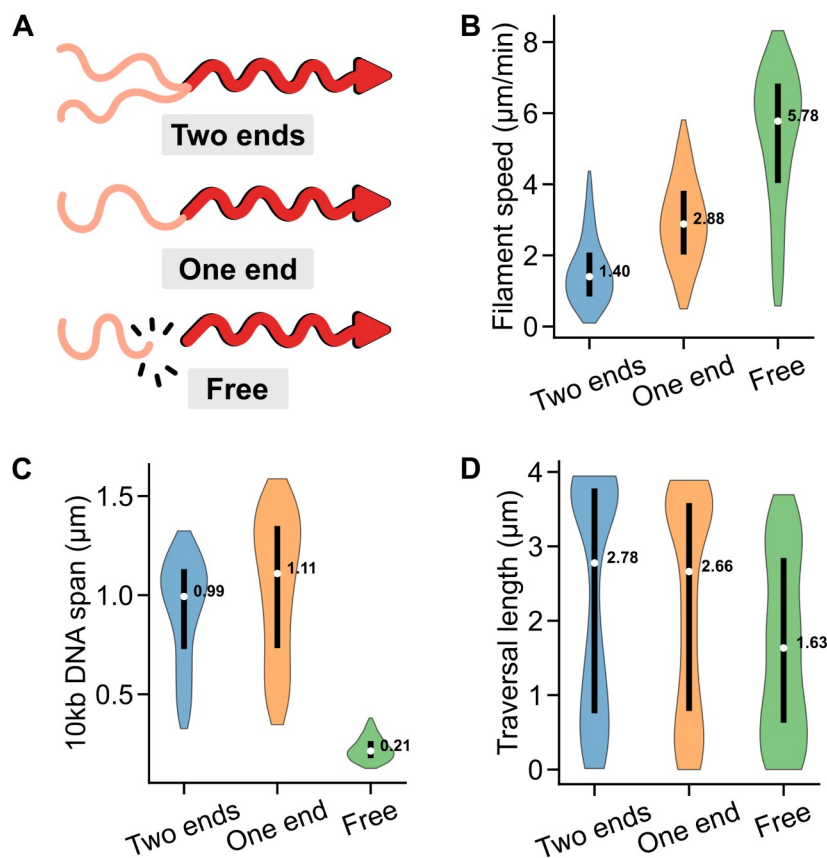


Figure 2.7: Effect of DNA segments attached to the tail of the filament. (A) Illustration of possible configurations for DNA segments attached to the tail of the filament. There can be either 2 ends of the DSB site attached to the filament or just 1 or none. (B) Distribution of filament speed for 3 cases as mentioned, highlighting the median and interquartile range of the distribution. (C) Distribution of long axis span of the 10kb DNA present next to the filament tail for 3 cases as mentioned, highlighting the median and interquartile range of the distribution. For the free filament case the span is measured from the 10kb DNA with the same spatial coordinates as in the other 2 cases. (D) Distribution of traversal length (highlighting the median and interquartile range) for 3 cases as mentioned, traversal length is measured as the displacement along the long axis of the cell made by the filament before reversing its

direction along the same axis of the cell. In B,C, and D the measurement was made only when filament is 0.5 μm away from the two poles of the cell, to avoid the effect of cell poles on the measured quantity.

Consistent with this, filament motion also leads to significant deformation of the attached DNA. As shown in Fig. 2.7C, the $\sim 10\text{kb}$ region which is adjacent to the RecA filament gets stretched up to ~ 4 times when filament is attached. This deformation is likely to contribute to the observed drag, as stretching a polymer such as DNA is entropically unfavorable and requires additional force to sustain motion (Rubinstein & Colby, 2003; S. B. Smith et al., 1992). Altogether these results combined with estimated total force generated by the RecN motors are in agreement with a recent experiment where ~ 1 pN of force was sufficient to drag a DNA loci up to several micrometers within minutes in the eukaryotic cell nucleus (Keizer et al., 2022).

Notably, this DNA-imposed resistance is not purely detrimental but also appears to play a functional role in sustaining directed motion. To quantify this, I measured the distribution of traversal lengths, defined as the distance traveled by the filament before reversing direction (Fig. 2.7D). Although the free filament moves faster, it exhibits significantly shorter traversal lengths. In contrast, the presence of trailing DNA leads to longer, more sustained traversals, suggesting that DNA-mediated drag stabilizes filament orientation and promotes unidirectional motion.

2.4 Filament rigidity governs its long range motion

The high bending rigidity of the RecA nucleoprotein filament is one of its defining mechanical properties and has been proposed to facilitate homology search by maintaining DNA in an extended, mechanically organized state. This extended and rigid conformation is also thought to promote interactions with DNA segments located at distant positions within the cell (Lesterlin et al., 2014; Wiktor et al., 2021). I therefore hypothesized that filament stiffness is critical for maintaining directional persistence during long range translocation. To test this, I quantified the translocation length (as described in the previous section) across simulations in which the persistence length of the RecA filament was varied from 50 nm to 2 μm (Fig. 2.8A). At very low persistence lengths (50-200 nm), long traversals, comparable to the cell length, were abolished, indicating that low stiffness is detrimental for long-range filament motion. At intermediate stiffness (0.5 μm), only a few long-range traversals are observed, with the majority being short, as reflected by a median traversal length of 0.78 μm . In contrast, at experimentally observed and higher persistence lengths (1-2 μm), at least 50% of traversals span the cell length. These results highlight the importance of filament stiffness in enabling

long-range, directed motion. This behavior can be attributed to increased bending fluctuations at low stiffness, which lead to frequent reorientation of the filament and disrupt directional persistence, whereas higher stiffness suppresses these fluctuations and enables sustained motion.

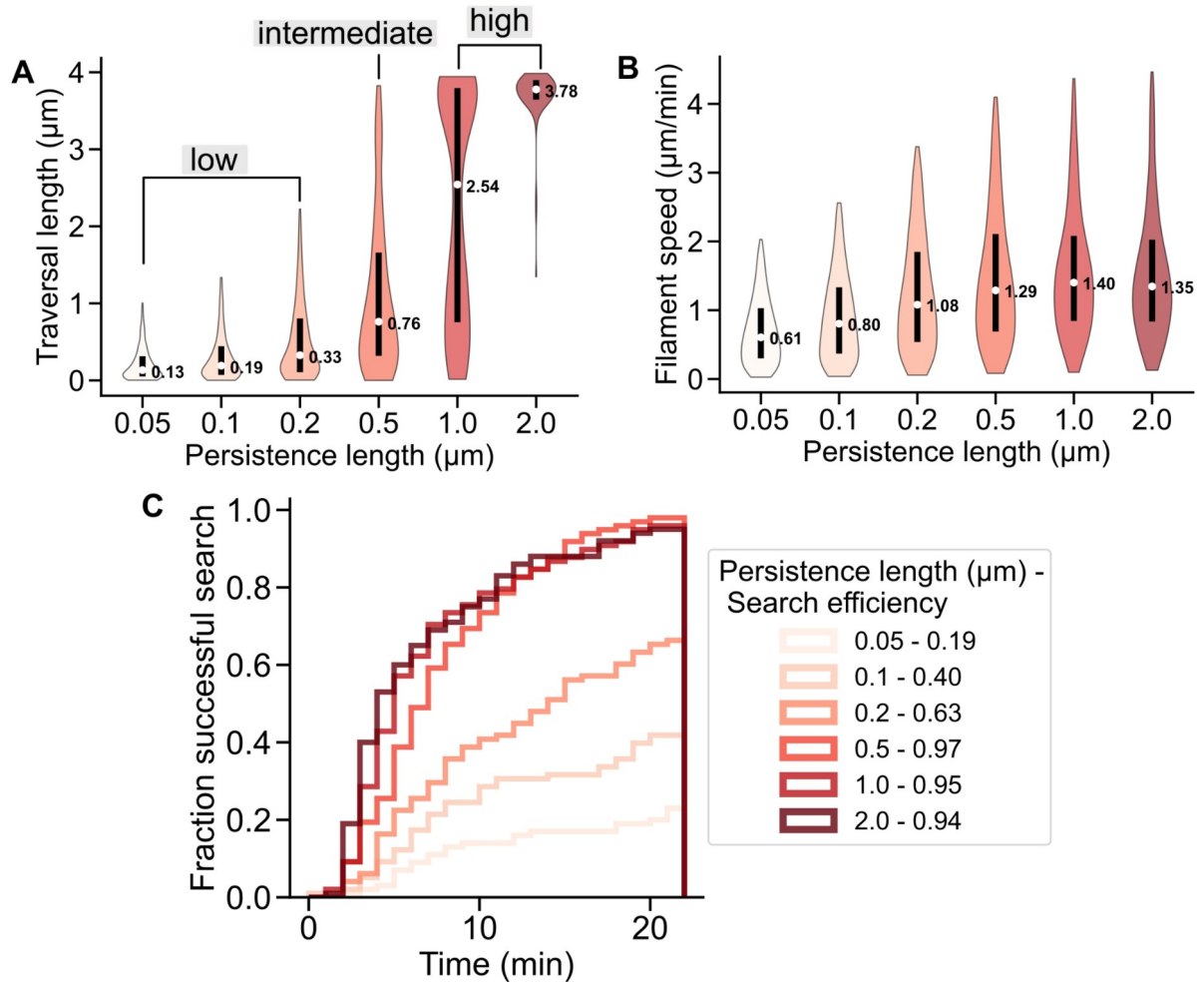


Figure 2.8: Effect of RecA filament stiffness on its motion and homology search efficiency. (A) Distribution of traversal length of the RecA filament with varied filament stiffness while keeping other parameters as constant. The filament stiffness is measured in persistence length (for details see Methods), which is distributed into 3 categories as low (0.05, 0.1 μm), intermediate (0.5 μm), and high (1.0, 2.0 μm). The median and interquartile range is highlighted in each case. **(B)** Distribution of filament speed for the different cases of filament stiffness. **(C)** Homolog search efficiency for the different cases of filament persistence length, the inset on the right highlights the persistence length of the given curve and its measured search efficiency.

In addition to directional persistence, filament rigidity may also influence the speed of translocation, as shown in Fig. 2.8B. The filament velocity decreases gradually with decreasing stiffness, indicating that rigidity supports efficient collective motor action. This reduction in speed may arise from increased bending at low stiffness, which introduces local variations in filament orientation. Consequently, motor activity is likely distributed along the

misaligned directions, reducing the net forward component of the motion. However, this effect on speed remains relatively modest compared to its much stronger impact on directional persistence.

Finally, search efficiency was quantified to assess the impact of stiffness on homology search (Fig. 2.8C). As expected, simulations with high persistence length (1-2 μm) exhibited high search efficiency ($\sim 95\%$). Interestingly, the efficiency remained similarly high ($>95\%$) even at intermediate stiffness (0.5 μm), indicating that once the filament is able to complete a traversal, successful target recognition is highly likely. However, the search is somewhat slower at intermediate stiffness, likely due to the reduced filament velocity. At low persistence lengths, the search efficiency drops significantly ($<60\%$), demonstrating that filament stiffness is critical not only for sustaining long-range traversals but also for efficient homology search. Taken together, these results show that filament rigidity primarily governs the stability and persistence of motion, enabling weak RecN-driven forces to produce efficient, long-range homology search at the cellular scale.

2.5 Free filament tip is required for sustaining unidirectional motion

While filament rigidity strongly influences the stability of motion, sustained unidirectional translocation also depends on how the filament is attached to the DNA ends. Because the filament remains connected to the DNA ends generated by a DSB, these attachments may constrain its ability to move directionally. Although a double-strand break generates two DNA ends, experimental observations typically reveal a single RecA filament, suggesting that the two ends remain associated during homology search (Chimthanawala et al., 2022; Lesterlin et al., 2014; Wiktor et al., 2021). This constraint limits the system to two possible configurations: a pendant configuration, in which both DNA ends are attached to the same trailing end of the filament, and a bridge configuration, in which the two DNA ends are attached to opposite ends of the filament, as illustrated in Fig. 2.9A.

From previous simulations we already know that the pendant configuration results in robust cell pole-to-pole RecA filament translocations. Therefore, I next examined the filament dynamics in bridged configuration. In these simulations, the filament exhibits circular motion and remains localized near its initial position (Fig. 2.9B). To quantify this effect, I compared the traversal length in both cases (Fig. 2.9C), and observed a ~ 15 fold reduction. This behavior is likely due to the attachment of DNA at both ends, which imposes curvature on the filament

and leads to continuous deflection of the filament tip, resulting in circular trajectories. In contrast, the filament speed is only weakly affected (Fig. 2.9D), indicating that motor activity remains largely intact. Together, these results suggest that in cells, the two DNA ends are more likely to remain associated in a pendant-like configuration to enable sustained unidirectional motion.

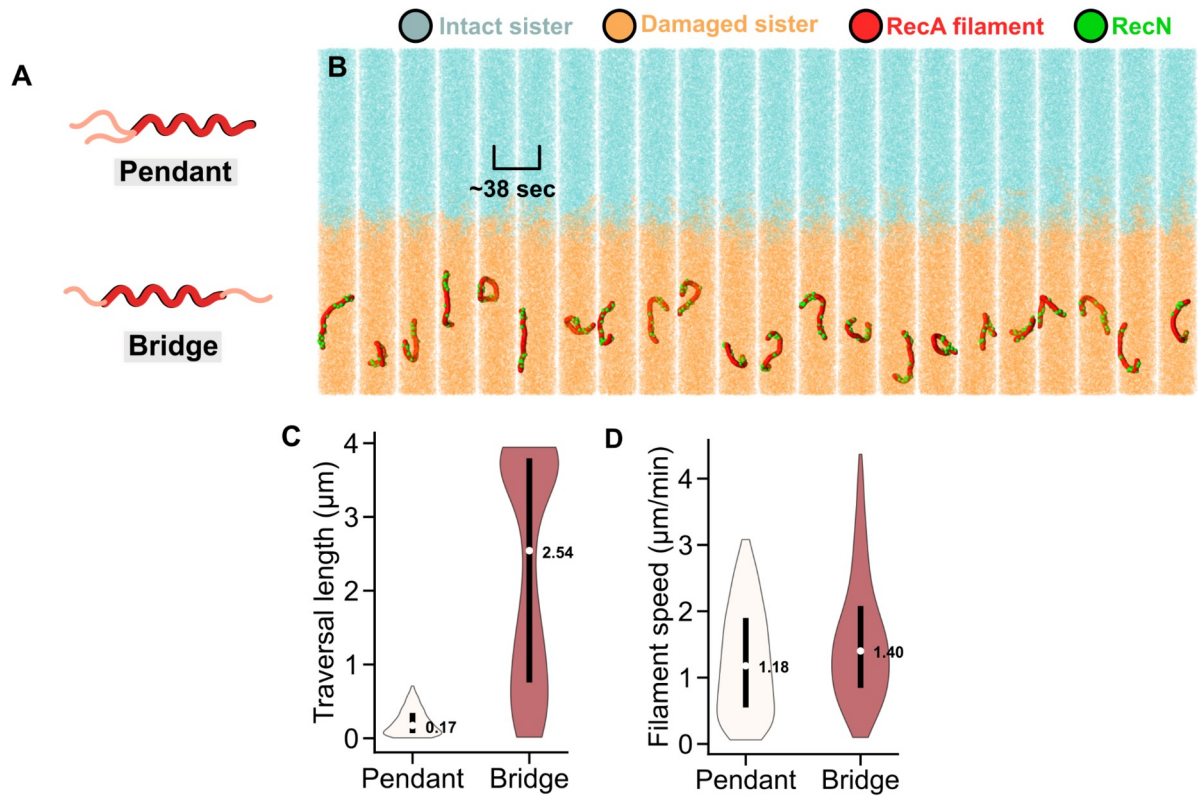


Figure 2.9: Effect of DNA attachment at different ends of the RecA filament on its motion. (A) Illustration of the 2 possible configurations for the 2 DNA ends to attach to the filament, namely, Pendant - when both ends are attached to the tail of the filament, and Bridge - when 2 ends are attached to the 2 opposite ends of the filament. **(B)** Simulation trajectory for the Bridge filament configuration, highlighting the circular motion in this case. **(C)** Traversal length comparison between Bridge and Pendant configurations, with their median and interquartile range. **(D)** Filament speed comparison between Bridge and Pendant configurations, with their median and interquartile range.

2.6 Distribution of RecNs on the filament affects its motion

I next examined how the spatial distribution of RecN motors along the RecA filament influences its ability to sustain unidirectional motion. In previous simulations, RecN particles were assumed to be uniformly distributed along the filament. However, experimental studies have shown that RecN often forms discrete DNA- or nucleoid-associated foci after DNA damage, rather than appearing as a uniform signal (Keyamura et al., 2013; Kidane et al., 2004). This suggests that localized clustering of RecN may be functionally relevant. For example, tail-localized RecNs may allow increased filament tip fluctuations, leading to reduced

directional motion. To test this, I compared three configurations in which the same number of RecN particles were localized adjacently at the tip, center, or tail of the filament, along with the randomly distributed, illustrated in Fig. 2.10A.

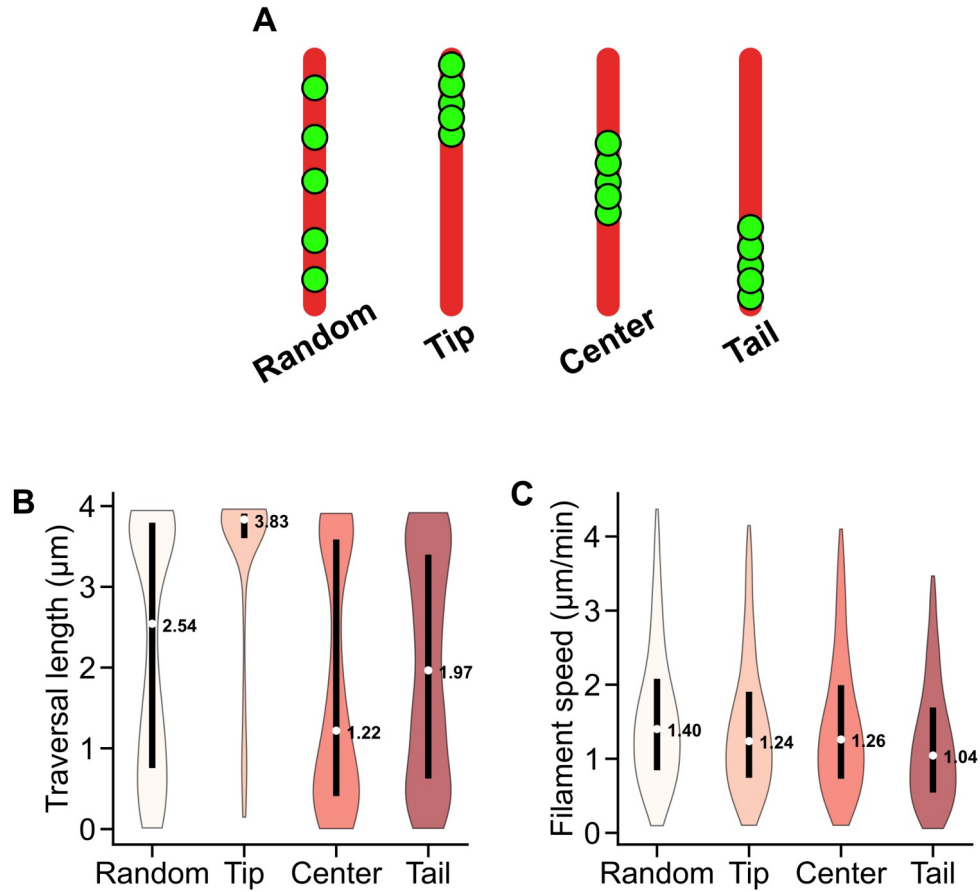


Figure 2.10: Effect of RecN localization on RecA filament motion. (A) Illustration of different localizations of RecN (green) on RecA filament (red). (B) Traversal length comparison between different RecN localization patterns, with their median and interquartile range. (C) Filament speed comparison between different RecN localization patterns, with their median and interquartile range.

As shown by the traversal length distributions (Fig. 2.10B), tip-localized RecN leads to the fewest midcell reversals and the highest fraction of complete traversals. In contrast, both center- and tail-localized configurations exhibit more frequent midcell and short-range (spontaneous) reversals. Interestingly, the center-localized configuration performs worse than the tail-localized case. This difference may arise because center localization produces a bimodal behavior, with trajectories dominated by either long-range or very short spontaneous traversals, resulting in a lower median traversal length. In contrast, the tail-localized configuration is enriched in intermediate (midcell) traversals, leading to a slightly higher median despite reduced overall directionality. Overall, these results indicate that sustained

unidirectional motion requires the presence of motors near the filament tip. This condition is naturally satisfied in the uniform distribution, where motors are available along the filament, including near the leading edge.

I also examined how the spatial distribution of RecN affects filament speed (Fig. 2.10C). In contrast to traversal behavior, the differences in speed across configurations are relatively modest. The random distribution exhibits the highest average speed, followed by tip- and center-localized configurations, while the tail-localized configuration shows the lowest speed. Overall, these results indicate that motor distribution has only a limited effect on the magnitude of filament velocity. Instead, its primary impact appears to be on the stability of motion, as reflected in the pronounced differences in traversal lengths and reversal behavior.

2.7 Filament translocations enable efficient cell volume exploration

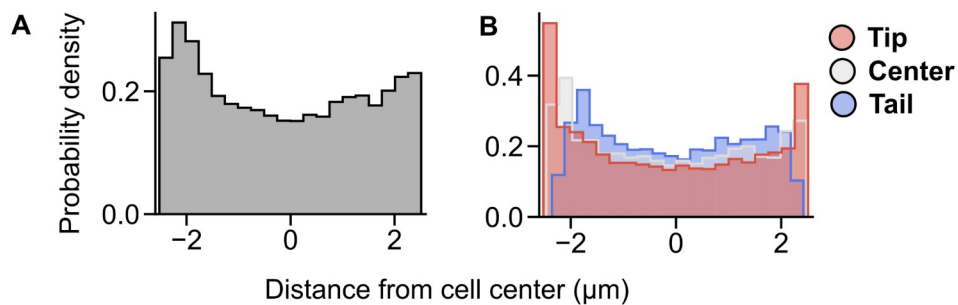


Figure 2.11: RecA filament explores the longitudinal axis of the cell efficiently. (A) Distribution of filament coordinates along the longitudinal axis of the cell. (B) Longitudinal distribution of the different parts of the filament, namely tip (red), center (gray) and tail (blue), (first, middle and last 10 monomers of the filament, respectively).

After identifying the key factors that influence RecN-assisted RecA filament motion, I next quantified the spatial distribution of filament positions within the bacterial cell. While the pole-to-pole traversals described above indicate efficient exploration along the longitudinal axis, they do not reveal how uniformly this space is sampled. I therefore computed the distribution of filament-position projections along the longitudinal axis of the cell (Fig. 2.11A). The filament coordinates span the entire cell length with noticeable enrichment at the cell poles. This enrichment reflects the time spent by the filament at the poles during directional reversal. To identify which part of the filament contributes most strongly to this enrichment, I analyzed the positional distributions of the filament tip, middle, and tail along the longitudinal axis (Fig. 2.11B). The strongest polar enrichment is observed for the filament tip, indicating that the leading end spends the most time near the poles. This behavior can be explained by the

reversal process: the tip reaches the pole first, while the remainder of the filament continues to advance. Only after the filament body approaches the pole does translocation resume in the opposite direction.

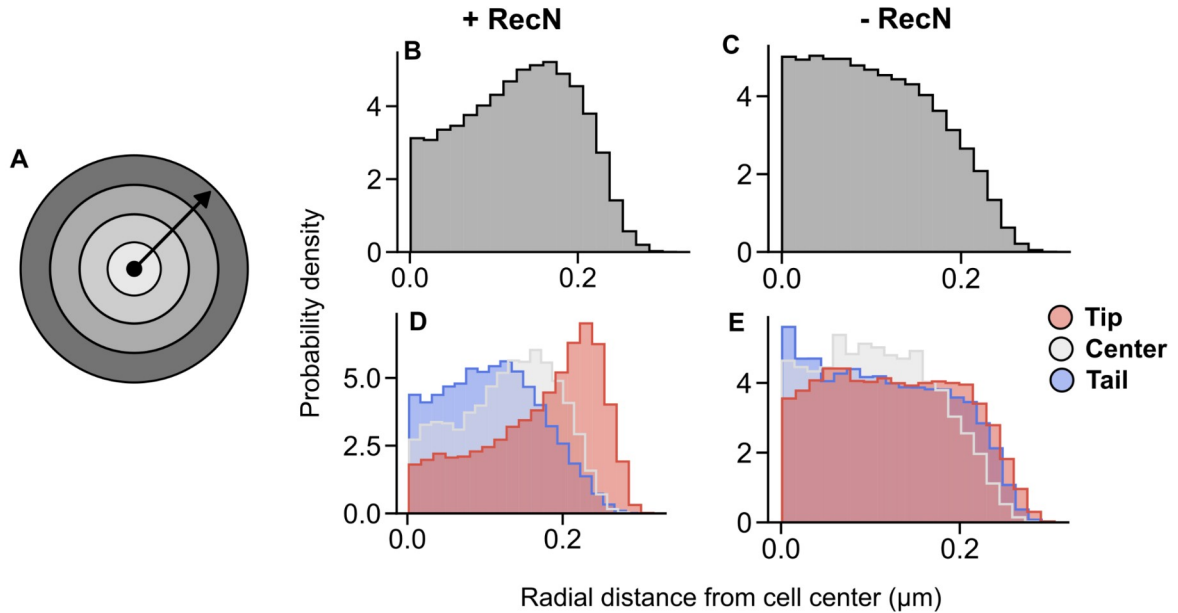


Figure 2.12: RecA filament explores the radial axis of the cell efficiently. (A) Illustration for the measurement of the filament coordinates along the radial cell space. (B,C) Distribution of filament coordinates along the radial dimension of the cell, with and without RecN, respectively. (D,E) Radial distribution of the different parts of the filament, namely tip (red), center (gray) and tail (blue), (first, middle and last 10 monomers of the filament, respectively), with and without RecN, respectively.

Next I examined how effectively the filament explores the cell's radial (cross-sectional) space. To quantify this, I computed the radial distribution of filament coordinates from the cell center, correcting for geometric bias arising from the larger available volume at higher radii, illustrated in Fig. 2.12A. The filament spans the whole radial extent of the cell, including the nucleoid region where most DNA is located (Fig. 2.12B). However, the distribution is shifted away from the cell center, with a median radial position of $\sim 0.15 \mu\text{m}$. To determine whether this bias arises from active motion, I compared this distribution to that of filaments lacking RecN (Fig. 2.12C). In the absence of RecN, the filament is most enriched at the center of the cell and gradually decreases toward the boundary, consistent with passive diffusion. This contrast indicates that the outward bias is a consequence of RecN-driven activity.

I next asked whether different parts of the filament exhibit distinct radial preferences. Similar to the longitudinal analysis, I computed radial distributions for the filament tip, middle, and tail (Fig. 2.12D). The filament tip is preferentially localized toward the cell periphery, whereas the

tail is more enriched within the central DNA-rich region. This spatial separation is absent in filaments lacking RecN (Fig. 2.12E). Together, these results indicate that RecN activity drives the filament tip to preferentially explore the nucleoid surface, while the filament body samples the DNA-rich interior, enabling efficient exploration of the entire cell volume.

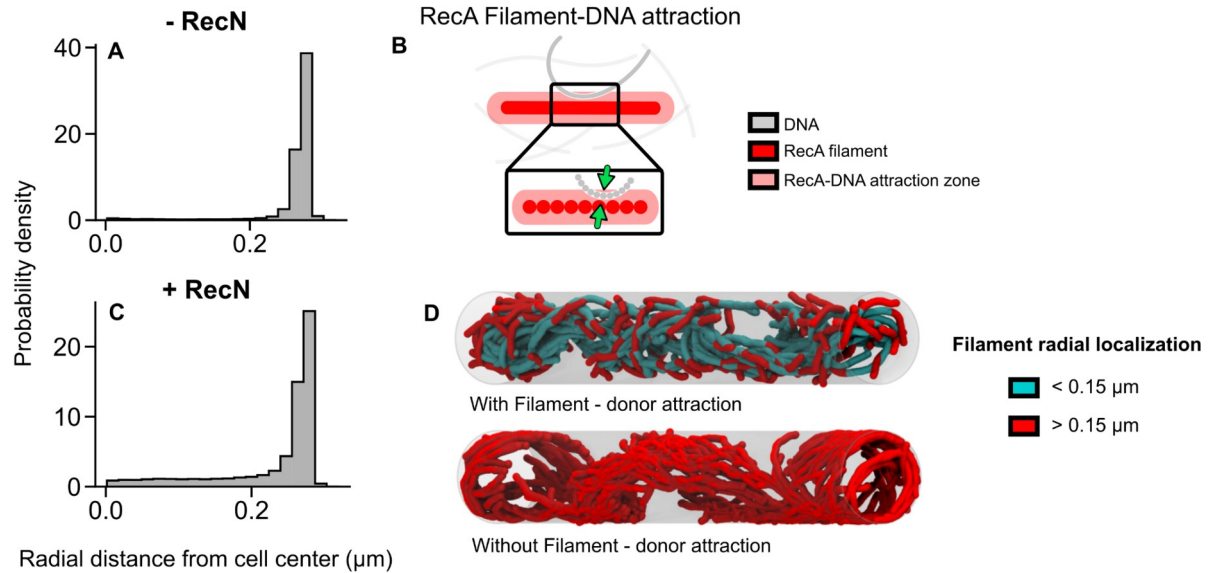


Figure 2.13: RecA Filament - DNA interaction enforces uniform radial cell space exploration. (A) Radial distribution of the filament coordinates in absence of the short range non-specific Filament - DNA attraction, in absence of RecN. (B) Schematic for the RecA - DNA short range attraction. (C) Radial distribution of the filament coordinates, same as A, but with RecNs. (D) RecA filament coordinates aggregated over the whole simulation trajectory, with near cell boundary and away from boundary coordinates labeled differently. Near boundary coordinates as $>0.15 \mu\text{m}$ from the cell center (colored in red) and away from boundary coordinates as $< 0.15 \mu\text{m}$ from the cell center (colored in cyan). Top: with filament DNA attraction, bottom: without filament DNA attraction.

To further characterize filament behavior, I examined which factors govern its spatial localization within the DNA-rich interior of the cell. To address this, I first considered static RecA filaments in the absence of filament-DNA attraction, since this attraction represents a key interaction between the filament and DNA. In this case, the filament consistently localizes near the cell boundary (Fig. 2.13A), indicating exclusion from the polymer-rich interior. This behavior is consistent with the well-established tendency of stiff polymers to be depleted from dense polymer environments due to entropic effects, as configurations within the crowded interior are energetically unfavorable compared to those near the boundary (Chauhan et al., 2021; Yethiraj et al., 1994), for details see Methods section 4.1.9. To account for this, I introduced a weak non-specific attraction between RecA and DNA monomers (Fig. 2.13B). This interaction restores the radial distribution of the filament to that observed previously (Fig. 2.13C), with significant localization within the DNA-rich region.

Importantly, this effect persists in simulations with RecN: in the absence of filament-DNA attraction, the filament remains confined to the periphery, whereas inclusion of the interaction allows it to sample both the bulk and boundary regions (Fig. 2.13C). This behavior is further illustrated by trajectory traces (Fig. 2.13D), where segments near the boundary ($> 0.15 \mu\text{m}$) are shown in red and those in the interior ($< 0.15 \mu\text{m}$) in cyan. Without attraction, the filament remains almost entirely at the periphery, whereas with attraction it explores both regions. Together, these results demonstrate that weak non-specific filament-DNA interactions are essential for maintaining filament localization within the nucleoid and enabling effective exploration of the cellular DNA.

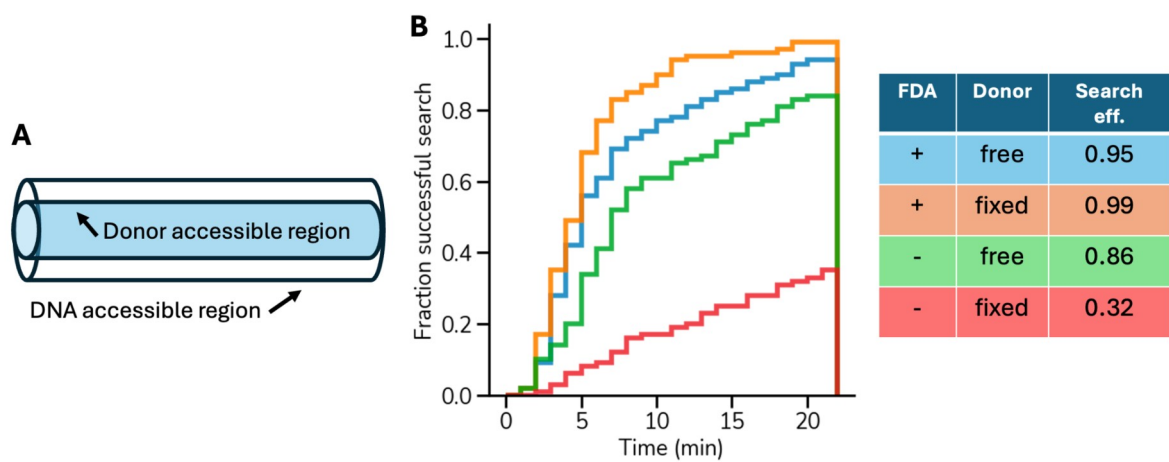


Figure 2.14: Restricted radial diffusion of donor DNA limits search efficiency when filament - DNA attraction is absent. (A) Illustration highlighting donor accessible regions in the cell in proposed simulations. (B) Homolog search efficiency when filament-DNA attraction (FDA) is present/absent and donor radial diffusion is restricted/free, inset table shows the search efficiency for respective combinations.

Having characterized filament localization, I then examined how this localization influences homology search efficiency. Efficient search is expected to depend on how thoroughly the filament explores the nucleoid, particularly if donor loci are not uniformly accessible. In standard simulations, the donor locus is freely diffusive, effectively sampling the entire cell volume. Under these conditions the search efficiency remains high ($\sim 86\%$, Fig. 2.14B) even in the absence of filament-DNA interaction, despite the filament being largely excluded from the nucleoid interior. However, the search process is slower, as indicated by a reduced cumulative frequency compared to the case with interaction. To test the importance of bulk exploration more directly, I considered a scenario in which the donor locus is spatially constrained. Specifically, I restricted donor diffusion to a small cylindrical region within the cell (Fig. 2.14A), mimicking a heterogeneous or locally confined chromosomal environment. Under these conditions, search efficiency drops sharply to $\sim 40\%$ in the absence of filament-DNA interaction, whereas it remains high when the interaction is present.

These results indicate that while surface exploration may suffice when the donor is freely accessible, efficient homology search in more realistic, spatially constrained environments requires the filament to sample the nucleoid interior. This framework may also help reconcile previous experimental observations in which RecA bundles or filaments were reported either outside or within the nucleoid. In cases where filaments are excluded from the nucleoid, a lack of effective interaction with DNA may impair bulk exploration and lead to slower search dynamics, consistent with reported search times of ~50 min (Lesterlin et al., 2014).

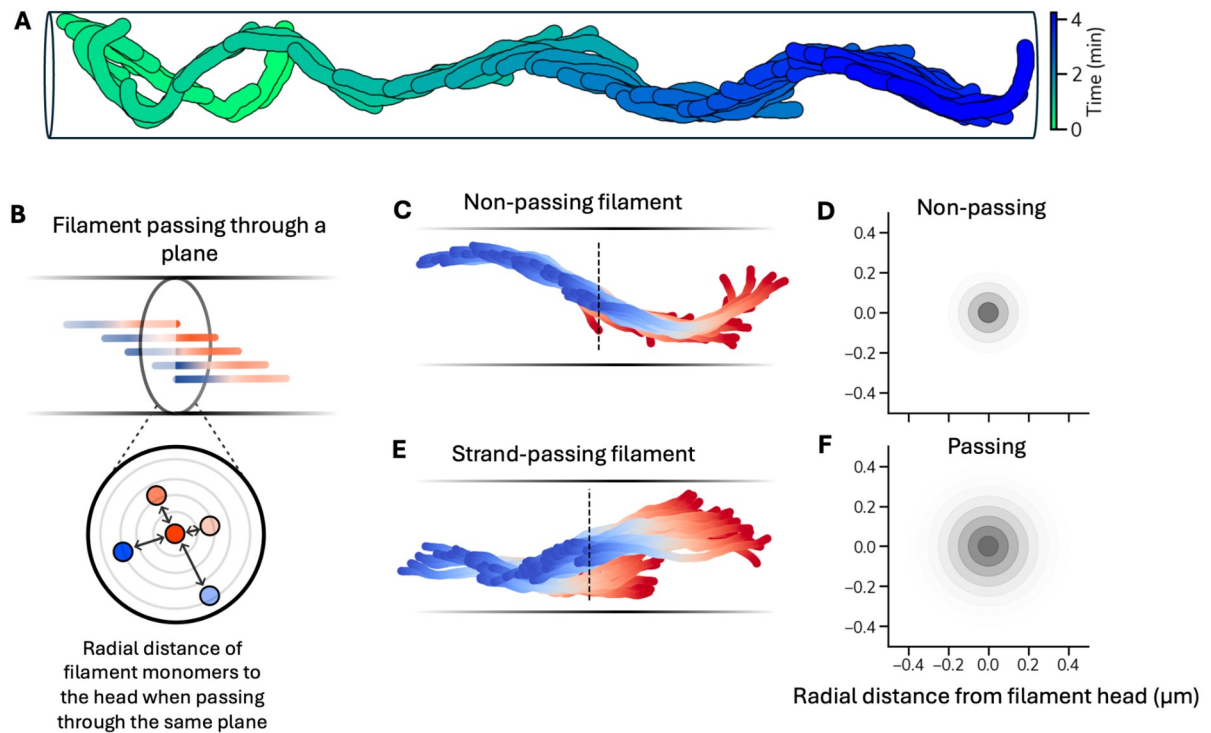


Figure 2.15: RecA filament showing reptation like behavior. (A) RecA filament coordinates aggregate over multiple frames, showing snake-like motion of the filament. (B) Scheme for determining the cross sectional area scanned by the filament while moving across a plane in the cell. (C) Example of RecA filament moving across such a plain, when filament passing is not allowed, colors on the filament highlight the different regions of the filament and their localization in each frame. (D) Distribution of the radial distance of different parts of the filament from its head when they are crossing the same plane (as illustrated in B), when filament passing is not allowed. (E) Example of RecA filament moving across such a plain, when filament passing is allowed, colors on the filament highlight the different regions of the filament and their localization in each frame. (F) Distribution of the radial distance of different parts of the filament from its head when they are crossing the same plane (as illustrated in B), when filament passing is allowed.

Lastly, I assessed how topological constraints arising from the surrounding DNA influence filament motion. In particular, I asked whether restricting the filament from passing through DNA strands alters its mode of translocation and its ability to sample local regions of the cell.

I observe that when strand passage is restricted, the filament exhibits a snake-like motion (Fig. 2.15A), in which the body of the filament follows the path traced by its leading tip. This behavior is reminiscent of the reptation dynamics of polymers moving through an entangled environment, where motion is constrained to a tube-like region defined by surrounding chains (Rubinstein & Colby, 2003). In our system, this arises because the filament cannot cross DNA strands and must instead navigate through the existing DNA mesh. To quantify this effect, I measured the effective area sampled by the filament body as it passes through a given cross-sectional region, as illustrated in Fig. 2.15B. When strand passage is restricted, the filament traverses a narrow spatial region, consistent with constrained, path-following motion (Fig. 2.15C,D). In contrast, when strand passage is allowed, the filament explores a broader area and the body no longer follows the trajectory of the tip (Fig. 2.15E,F). These results suggest that reptation-like motion enables the entire filament to repeatedly sample the same spatial path, allowing different segments of the filament to probe nearby DNA sequences for homology. This constrained mode of motion may therefore enhance local search efficiency by ensuring thorough sampling of regions encountered by the filament tip.

2.8 RecN motor action can speed up homology search in other bacteria

So far, the current model has established a potential mechanism for RecN induced RecA filament cell pole-to-pole translocations in *C. crescentus*, and characterized the factors affecting this motion and homolog search efficiency. However, it is still unclear if RecN performs similar functions in other bacteria or if there are other RecN dependent mechanisms which do not involve such large-scale motion. RecN is a highly conserved SMC-like bacterial protein that contributes to homologous recombination-mediated DSB repair (Ayora et al., 2011; Chimthanawala et al., 2022; Funayama et al., 1999; Kosa et al., 2004; McLean et al., 2021; Wang & Maier, 2008). Therefore next I sought to investigate if such motor-like motion is crucial for DSB search in other bacteria.

In *E. coli*, fluorescence imaging of tagged RecA revealed the formation of extended filaments that span the cell and undergo dynamic motion (Wiktor et al., 2021). Based on these observations, it was proposed that homology search proceeds via a reduced-dimensionality mechanism. In this model, the RecA filament extends across the cell due to its high stiffness, effectively reducing the search from three dimensions to a two-dimensional process. The donor DNA is then thought to diffuse toward the filament and encounter it through random collisions, enabling homology recognition. Such a mechanism has been suggested to allow

target search within a timescale of ~ 10 minutes. However, this framework relies on key assumptions, namely that the filament can extend across the cell through diffusion and intrinsic stiffness alone. I therefore evaluated the efficiency of this reduced-dimensionality search mechanism under conditions relevant to the *E. coli* cell.

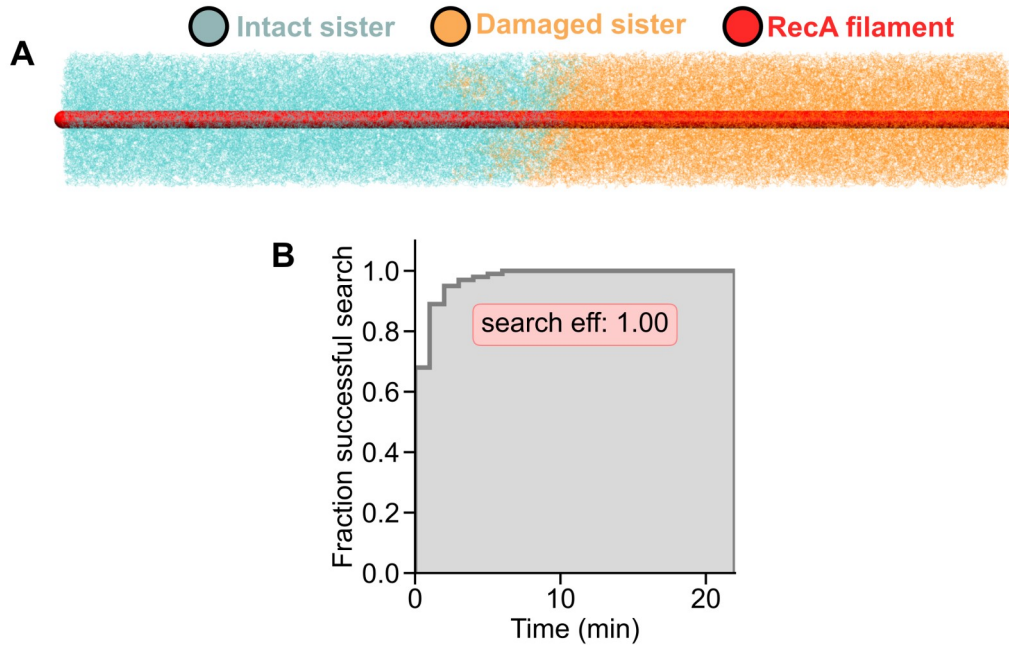


Figure 2.16: Simulation of reduced dimensionality model for *E. coli* cell. (A) Simulation model for *E. coli* where cell length long filament is centrally located in the cell (for time units conversion see Methods section 4.1.2). (B) Homology search efficiency for reduced dimensionality search model.

I modified the *C. crescentus* model with respect to the cell and genome size of a typical *E. coli* cell. Since *E. coli* cells are a bit shorter than the *C. crescentus*, I set its length to $4\ \mu\text{m}$ only, keeping the same diameter. But since its genome is slightly larger ($\sim 4.6\text{Mbp}$), each sister length was also increased to 154666 monomers (keeping the coarse-graining same at 30bp per monomer). Similarly, DNA diffusion was also calibrated with respect to the DNA dynamics in the real bacterial cell. However, the filament properties, including its stiffness and interaction with DNA, were kept unchanged. The only parameter modified was the filament length, which was set equal to the cell length ($4\ \mu\text{m}$).

To test the search efficiency for the suggested model I artificially extended and fixed the filament at the center of the cell along the long axis, as shown in Fig. 2.16A. Then I evaluated the search timings, and in most of the simulations the search finished within 5 min (Fig. 2.16B), as proposed by the model. Next I tested if a freely diffusing cell length long RecA filament can expand through the cell due to its stiffness, and achieve similar search efficiency. In most simulations, the filament failed to expand throughout the entire cell, even in 30 min, as shown

in one of the trajectories in Fig. 2.17A. As a result the search efficiency was only ~30% (Fig. 2.17B). These results suggest that passive diffusion alone is insufficient to explain the fast homology search observed in-vivo. Therefore there must be some active mechanism involved to extend the filament further.

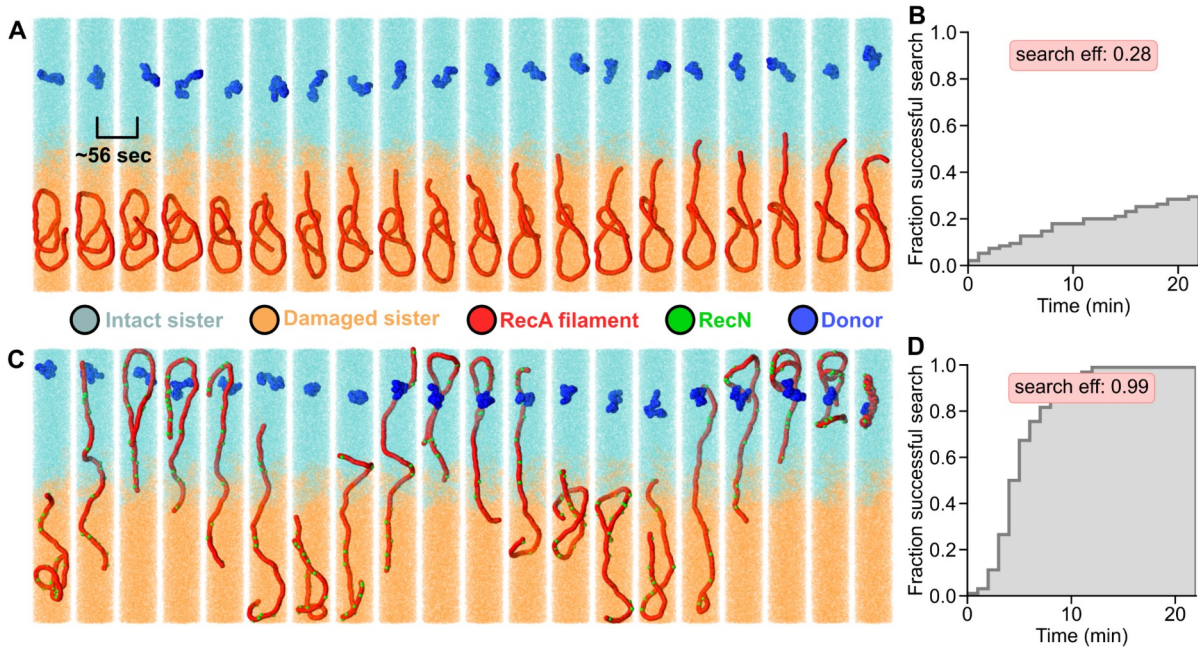


Figure 2.17: Simulation with cell length long RecA filament in *E. coli* cell. (A) Simulation trajectory with freely diffusing cell length long filament, with filament - donor pairwise attraction enabled (for time units conversion see Methods section 4.1.2). **(B)** Homology search efficiency for the *E. coli* simulation without RecN. **(C)** Simulation trajectory with RecNs associated cell length long filament, with filament - donor pairwise attraction enabled. **(D)** Homology search efficiency for the *E. coli* simulation with RecN.

As mentioned earlier, RecN is essential for the DSB repair in *E. coli* as well. So next I asked if the motor-like action of RecN can help in extending the filament, within a reasonable time, leading to experimentally observed search timings. Therefore I included 20 RecNs, same as in *C. crescentus*, in the *E. coli* simulation, which were uniformly distributed on the filament. RecN motor properties were also kept the same, with the same parameters. In these simulations, RecA filament was able to extend throughout the whole cell length, similar to experimental observations (Fig. 2.17C). The search efficiency also rose to ~100% within 10 min of the simulation (Fig. 2.17D). Therefore these simulations suggest that motor-like motion of RecN can also help RecA filament to stretch and find the donor quickly in *E. coli* cells.

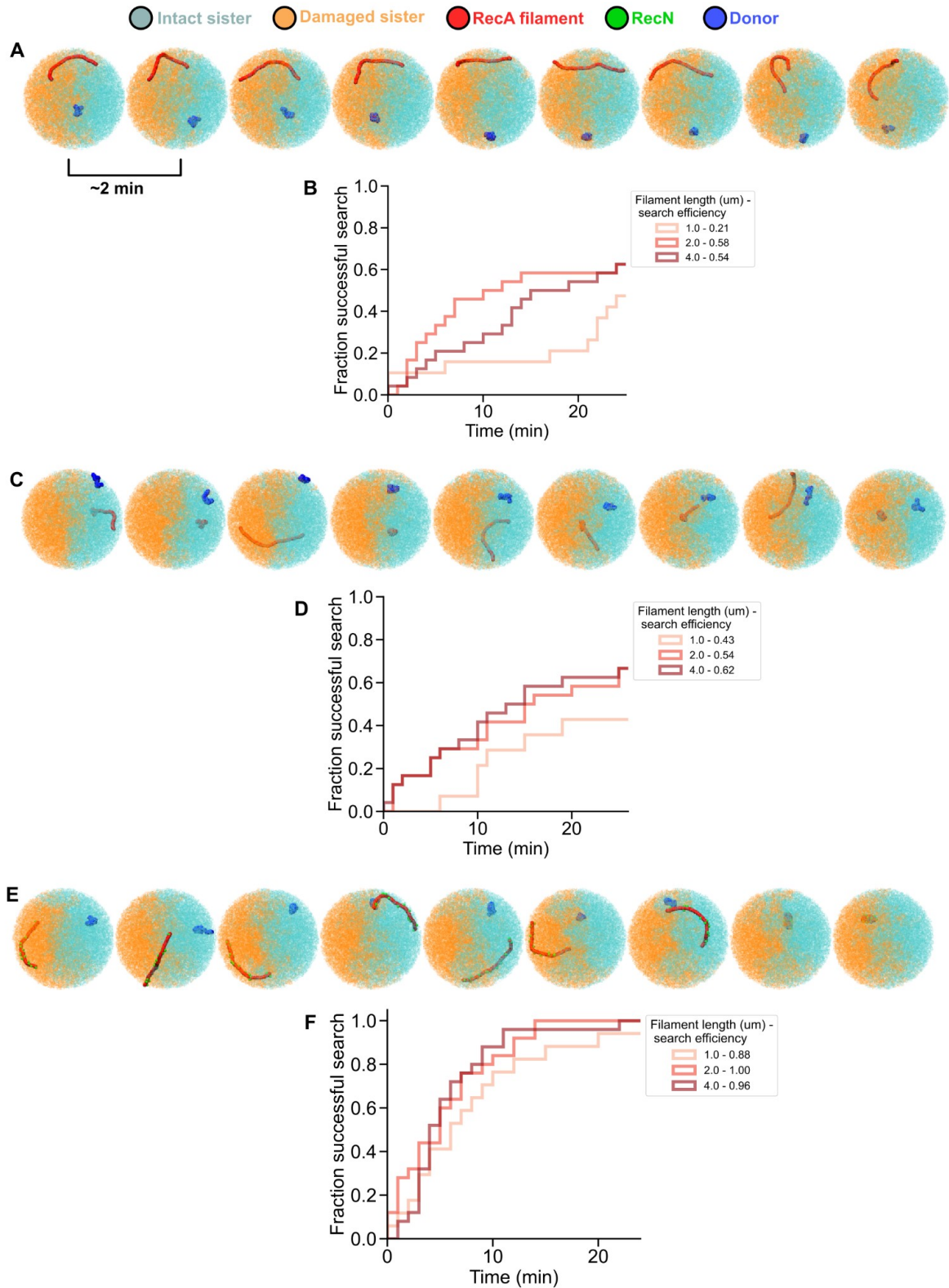


Figure 2.18: homology search simulation in an artificial spherical . (A) Few snapshots from simulation trajectory with freely diffusing 1 μm long RecA filament, with filament - donor pairwise attraction enabled (for time units conversion see Methods section 4.1.2). (B) Homology search efficiency for the simulations in the spherical geometry with different filament lengths (1, 2, and 4 μm). (C) Few snapshots from simulation trajectory with freely actively shrinking and expanding 1 μm long RecA filament, with filament - donor pairwise attraction enabled. (D)

Homology search efficiency for the simulations in the spherical geometry with actively shrinking and expanding filaments of different lengths (1, 2, and 4 μm). **(E)** Few snapshots from simulation trajectory with RecN associated 1 μm long RecA filament, with filament - donor pairwise attraction enabled. **(F)** Homology search efficiency for the simulations in the spherical geometry with RecN associated filaments of different lengths (1, 2, and 4 μm).

Lastly, I wanted to check if the role of RecN is cell geometry independent, as there are many bacteria which have spherical or arbitrary shapes. Irrespective of that, RecN is present in spherical bacteria as well. To test that, I constructed an artificial model with spherical cell geometry, DNA content, and density same as *C. crescentus* cells. In these spherical cell models, the final sphere diameter was $\sim 1.4 \mu\text{m}$, which is comparable to many spherical bacteria and yeast nuclei. Initially, similar to *E. coli*, I simulated freely diffusing filament, as shown in Fig. 2.18A. However, the results were similar to *E. coli*, with low search efficiency of $\sim 50\%$ (Fig. 2.18B). Then I also tested with larger filaments, with length comparable to cell diameter and more (2 and 4 μm). In both cases search efficiency increased, but only slightly, up to $\sim 60\%$. Therefore in spherical geometry too, a passively diffusing filament is insufficient for homology search.

Recently in yeast, a semi-active mechanism was proposed to help in homology search where the Rad51 filaments shrink and expand multiple times leading to enhanced cell volume exploration (S. Liu et al., 2023). So I implemented a similar mechanism to see if that can increase the search efficiency in our simulations (Fig. 2.18C). However, the search efficiency was still similar to passive filaments, being highest for longer filaments and up to $\sim 60\%$ (Fig. 2.18D). Thus it appears that such semi-active diffusion based mechanism is still insufficient for homology search in spherical cells, for similar time scales. However, for yeast such a mechanism may work as it takes hours for the whole repair process.

Finally, I added RecNs to the RecA filament in these simulations. The RecA filament showed rapid motion throughout the whole cell as shown in the trajectory snapshots in Fig. 2.18E, and the search efficiency also increased to $\sim 100\%$, comparable to other bacteria, even for 1 μm long filament, Fig. 2.18F. Therefore these results show that through motor-driven strategy RecNs can facilitate faster homology search in spherical cells. Altogether the results in this work suggest that RecN's motor-like action can provide a universal solution for the homology search in various bacterial systems.

3. Discussion

Repair of DNA double-strand breaks (DSBs) is an essential process that all cells must carry out throughout their lifetime. Among the available repair pathways, homology-directed repair (HDR) is the most accurate because it restores genetic information using an intact homologous template. Although the biochemical framework of HDR has been extensively characterized in many organisms, the homology search mechanism itself remains a major physical challenge. In *Caulobacter crescentus*, RecA nucleoprotein filament was observed to undergo pole-to-pole translocations during the search process (Chimthanawala et al., 2022). These translocations are driven by the SMC-like protein RecN. In the present work, I sought to determine the mechanism by which RecN drives these large-scale filament translocations. Our results showed that a simple DNA motor-like activity associated with the RecA filament is sufficient to generate long-range directional motion. More broadly, the findings help explain why RecN may be so strongly conserved in bacteria despite the diversity of genome sizes and cellular geometries.

3.1 A minimal motor-like model explains RecN-driven RecA filament translocation

The experiments have already shown that RecN is essential for RecA filament translocation in *C. crescentus*, and that this process depends on the ATPase activity of RecN (Chimthanawala et al., 2022). ATPase mutants abolished filament motion, suggesting that RecN is not merely a passive scaffold, but rather an active component of the translocation process. Notably, in the ATP-binding mutant, filament length remained largely unaffected, yet translocations were lost. This indicates that the defect is not in filament assembly or stability, but in the ability to generate movement. Taken together, these results suggest that RecN plays an active mechanical role in filament translocation. Our simulations support this, showing that a simple ATP-dependent motor-like action on the RecA-DNA complex is sufficient to drive the long-range directional motion observed during homology search.

To represent this active role, I modeled RecN as a minimal force-generating motor acting at the interface between RecA filament and DNA in an ATP-dependent manner. In this simplified approach, RecN transiently binds a nearby DNA segment, undergoes ATP-dependent conformational change, displaces the bound segment, releases it and then returns to its initial state. This four-step cycle was intended to capture the essential mechanical consequences of

the ATP turnover rather than full molecular details of RecN activity. In physical terms, the motor displaces DNA in one direction, and because the motor is associated with the RecA filament, this DNA displacement produces motion of the filament in the opposite direction. Thus, local ATP-dependent DNA displacement is converted into large-scale RecA filament translocation.

This representation is motivated by the broader behavior of the SMC-family proteins, many of which act as ATP-dependent DNA motors. Canonical SMC complexes such as cohesin and condensin are often described through loop-extrusion mechanisms, in which ATP-driven conformational changes displace DNA and generate progressively larger DNA loops (Alipour & Marko, 2012; Davidson et al., 2019; Ganji et al., 2018). However, RecN differs from canonical loop-extruding SMC complexes in an important way: it lacks a known kleisin subunit that would allow stable entrapment of DNA. For this reason, I did not model RecN as a persistent loop extruder. Instead, I treated it as a transient DNA-displacing motor, capable of repeated DNA binding, force generation, and release. In this view, RecN shares the general SMC-like principle of ATP-dependent DNA displacement, but does not require the stable DNA entrapment characteristic of canonical loop extrusion.

Other microscopic implementations of RecN activity are possible. For example, RecN could be modeled as a factor that binds both RecA and DNA and directly pushes the RecA filament, rather than displacing DNA as in our simulations. Similarly, the motor cycle could be represented using different conformational states, such as transitions between a flexible and a structured configuration, instead of two well-defined bound and unbound states. These alternatives may differ in microscopic detail, but they would be expected to produce similar large-scale behavior if they generate a biased ATP-dependent force between the RecA filament and DNA. We therefore used the simplest realization of such a motor, because it isolates the key physical requirement: ATP-dependent force generation is sufficient to produce the observed RecA filament translocation.

A further assumption in the model concerns the polarity of RecN action. Productive translocation requires that RecN bind in an oriented manner relative to the RecA filament, such that repeated motor cycles generate coherent motion rather than random or opposing forces. This assumption is plausible because RecA forms a polar right-handed helical filament, with RecA monomers arranged in a defined orientation rather than randomly (Bell & Kowalczykowski, 2016b). Previous evidence that RecN interacts with RecA in a species-specific manner further suggests that RecN binding may depend on the structural organization of the RecA filament (Inoue et al., 2026; Uranga et al., 2017). Thus, although the precise

polarity of RecN relative to RecA remains to be determined experimentally, the model assumes that RecN binds in a conformation that favors forward filament motion. The number of active RecN motors then primarily controls the magnitude of the cumulative force and the resulting translocation speed, while the existence of directional motion depends on the polarity and coordination of individual motor actions.

3.2 RecN-driven motion makes homology search efficient

RecN-driven translocation makes homology search efficient by converting local motor activity into large-scale displacement of the RecA filament. This is important because the donor sequence may be located far from the DSB site. Local diffusion or small filament fluctuations would not reliably bring the filament into contact with distant chromosomal regions. In our simulations, RecN-driven motion allowed the filament to traverse the cell and explore the donor-containing region, thereby increasing the probability of productive encounter. Importantly, this motion was not simply rigid forward displacement: even within the rod-shaped confinement used to represent the *C. crescentus* cell, the filament was able to turn around at the poles and continue translocating. Thus, RecN-driven motion appears to make the search spatially productive by combining persistent cell-scale displacement with the ability to reorient within a confined bacterial geometry.

The search times obtained in the simulations should nevertheless be interpreted as an idealized lower bound. In many trajectories, the filament found the donor segment rapidly, with approximately half of the cells completing the search within a few minutes. These times are faster than those observed experimentally in *C. crescentus*, likely because the model excludes several factors that would slow search in-vivo, including RecA filament remodeling, ATP-hydrolysis-dependent local flexibility within the filament, filament-destabilizing interactions with accessory proteins such as RecX (Alekseev et al., 2022; Cox, 2007), and transient encounters with partially homologous DNA sequences. In-vivo, RecA filaments are not perfectly static structures: RecA monomers undergo ATP hydrolysis within the filament, which can locally alter filament stability and flexibility (J. Y. Lee et al., 2016; VanLoock et al., 2003; B. Zhao et al., 2017). Competition with SSBs may further destabilize or remodel the filament (Bell et al., 2012; Wu et al., 2017), while accessory factors such as RecX can interact with RecA and modify its local properties (Alekseev et al., 2022; Cox, 2007). In addition, RecN itself may transiently dissociate from the filament, which would reduce the effective motor activity and could slow or interrupt filament motion. Beyond these structural and motor-related effects, the search process may also be slowed by DNA sequence sampling. As the RecA filament scans

the chromosome, it is expected to encounter many partially homologous regions before locating the correct donor sequence, and such transient interactions could delay productive homology recognition.

Therefore, the simulated search times should not be interpreted as exact in-vivo repair times, but rather as a lower-bound estimate for an idealized system in which filament properties remain stable and only one exact donor sequence is available.

3.3 Filament stiffness enables persistent direction translocation

The ability of RecN motors to generate directional motion depends strongly on the mechanical properties of the RecA filament. In our simulations, robust pole-to-pole translocations were observed only when the filament had sufficiently high stiffness. This suggests that filament rigidity is not simply a structural property of the RecA nucleoprotein filament, but a functional requirement for converting local motor activity into coherent long-range motion. Since individual RecN motors act locally on DNA segments associated with the filament, their forces must be transmitted along the filament to produce movement of the entire search complex. A stiff filament can transmit these forces more effectively, whereas a flexible filament tends to dissipate motor activity into local bending and reorientation.

This was reflected in the increased frequency of directional reversals at lower persistence lengths. When the filament was flexible, it often changed direction before completing a full traversal, leading to shorter net displacement and reduced cell-scale motion. At very low persistence length, reversals became so frequent that complete pole-to-pole traversals were rarely observed. In this regime, RecN motors could still generate local movement, but the filament lacked the mechanical persistence required to maintain a stable direction over long distances. Thus, reduced stiffness did not necessarily eliminate motor activity, but it prevented that activity from being translated into sustained translocation.

The relationship between stiffness and filament speed also suggested a transition between force generation and directional stabilization. Filament velocity increased with stiffness at lower and intermediate persistence lengths, indicating that a more rigid filament couples more efficiently to RecN motor activity. However, at the highest tested stiffness, increasing the persistence length further did not substantially increase the translocation speed. Instead, the main effect was a reduction in reversals and a more stable pole-to-pole trajectory. This suggests that once the filament is stiff enough to transmit motor forces efficiently, additional

stiffness no longer increases the maximum motor output, but improves the persistence and reliability of motion.

Therefore, filament stiffness appears to play two related roles during RecN-driven homology search. First, it allows local ATP-dependent motor activity to be converted into global filament displacement. Second, it stabilizes the direction of motion, reducing stochastic reversals that would otherwise limit long-range exploration. This provides a physical explanation for why the high rigidity of RecA filaments may be important during homology search.

3.4 DSB-end geometry determine motion stability

The previous section shows that a stiff RecA filament is required to transmit RecN-generated forces over long distances. However, stiffness alone does not determine whether these forces will result in productive translocation. The same motor activity can lead to very different motion depending on how the broken DNA ends are connected to the filament. This is important because a double-strand break creates two DNA ends, yet experimental imaging often reports a single visible RecA filament during the search process (Chimthanawala et al., 2022; Wiktor et al., 2021). If the two ends separated completely, repair of a two-ended break would be difficult to coordinate. Therefore, we considered the possibility that both DSB ends remain associated with a common RecA-based search complex, even if they are not resolved as two independent filaments.

The assumption that the two DSB ends remain associated during homology search is consistent with recent studies in eukaryotes and bacteria. In budding yeast, DSB end-tethering limits separation of the two broken ends, and recent work suggests that coordinated search by opposite Rad51 nucleoprotein filaments is important for efficient repair (Dumont et al., 2024; Phipps et al., 2025; Piazza & Taddei, 2026). Thus, even when both DNA ends are processed, their search activity is spatially coordinated rather than completely independent. A similar interpretation is consistent with bacterial imaging studies, where a two-ended break is often visualized as a single extended RecA search structure rather than two clearly separated filaments (Chimthanawala et al., 2022; Lesterlin et al., 2014; Wiktor et al., 2021). This visible structure could represent one dominant RecA filament associated with both DNA ends, or two end-associated filaments that remain closely aligned and are not resolved as independent structures. We therefore modeled the two DSB ends as associated with a common RecA-based search complex and compared two limiting arrangements: a pendant configuration, in

which both ends are attached near the trailing side of the filament, and a bridged configuration, in which the ends are attached to opposite filament ends.

I found that only the pendant configuration supports sustained unidirectional motion. When both DNA ends are attached near the trailing side, the filament retains a free leading tip and undergoes robust pole-to-pole translocation. In contrast, attaching the two DNA ends to opposite filament ends constrains the filament, leading to curved trajectories and greatly reduced traversal lengths. Thus, coordinated DSB ends can support RecN-driven motion only when their arrangement preserves a free filament tip.

3.5 Biophysical plausibility of RecN-generated forces

The force analysis supports the biological plausibility of RecN-driven filament motion by placing the mechanism in a low-force regime consistent with SMC-like DNA motors. Importantly, the effective force transmitted to filament-scale motion should not be interpreted as the intrinsic force capacity of a single RecN molecule. Rather, it reflects the fraction of local motor activity that is productively converted into net filament displacement. This conversion can depend on transient DNA binding and release, filament stiffness, DNA tethering, and the geometry of force application. Therefore, the lower effective stall force measured at the filament level likely reflects mechanical filtering within the RecA-DNA system, rather than simply weak or inefficient motor activity.

This interpretation fits the broader model proposed here: RecN does not need to act as a strong individual motor to drive long-range translocation. Instead, robust motion can emerge from the cumulative action of many weak ATP-dependent motors acting on a stiff RecA-DNA complex. The attached DNA imposes an entropic load because stretching a polymer away from its coiled equilibrium state reduces conformational entropy and therefore requires force (Rubinstein & Colby, 2003). Consistent with this, our simulations show that the DNA trailing behind the filament is stretched during translocation, yet this load can be overcome by collective RecN activity. The required force scale is also compatible with recent micromanipulation experiments showing that near-piconewton forces are sufficient to displace genomic loci over micrometer distances within minutes in living nuclei (Keizer et al., 2022). Thus, the trailing DNA is not merely a passive obstacle: it slows the filament, but also helps stabilize directional motion by resisting rapid reorientation. In this way, weak RecN-generated forces can be converted into persistent filament translocation when applied to a stiff filament with an appropriately oriented DNA tether.

3.6 RecA-DNA interactions enable nucleoid exploration

RecN-driven motion can generate long-range filament translocation, but productive homology search also depends on where this motion occurs. In our simulations, removing weak nonspecific RecA-DNA attraction caused the stiff filament to be excluded from the DNA-rich nucleoid bulk and to move preferentially near the cell boundary. This is consistent with polymer-physics expectations, as stiff polymers can be depleted from dense flexible-polymer environments because their insertion imposes packing and conformational costs (Chauhan et al., 2021; Yethiraj et al., 1994). Thus, without RecA-DNA attraction, the filament may remain mobile but explore regions that are less relevant for locating chromosomal homology.

This distinction explains why the absence of RecA-DNA attraction did not strongly reduce search efficiency when the donor was freely diffusive: the donor could still reach the boundary-localized filament. However, when donor motion was restricted to the DNA bulk, filament exclusion strongly reduced search efficiency, showing that RecA-DNA attraction is important mainly because it keeps the searching filament within the chromosomal volume. This interaction is also biologically reasonable, since RecA filaments are known to transiently interrogate dsDNA during homology search before stable strand invasion (Ragunathan et al., 2012). Single-molecule and biochemical studies further suggest that RecA/Rad51-family recombinases sample short regions of homology, with stable pairing favored only after sufficient microhomology is detected, often around an 8-nucleotide threshold (J. Y. Lee et al., 2016; Qi et al., 2015). Therefore, the nonspecific RecA-DNA attraction in the model can be interpreted as a coarse-grained representation of weak, transient dsDNA probing before stable pairing.

This interpretation may also help explain why the RecA bundles observed by Lesterlin et al., 2014 were associated with the nucleoid periphery and showed slower long-range pairing dynamics. One possibility is that thick RecA bundles interact less effectively with the DNA-rich bulk than thinner filaments, leading to spatially less efficient search. Thus, efficient homology search requires both RecN-driven long-range motion and weak RecA-DNA interactions that localize this motion within the nucleoid.

3.7 RecN motor action may provide a general strategy across bacterial geometries

The results in *C. crescentus* suggest that RecN-driven motor activity can overcome an important physical limitation of homology search: the difficulty of moving a large RecA filament over cellular length scales by diffusion alone. To test whether this principle was specific to the asymmetric rod-shaped geometry of *C. crescentus*, we extended the model to other bacterial cell geometries. These simulations suggest that the advantage of RecN is not limited to one particular cell shape, but arises from a more general need to actively reposition the RecA filament within the cellular volume.

This was particularly evident in the *E. coli*-like system. Previous models have suggested that a long and rigid RecA filament could enhance homology search by extending through the cell and reducing the dimensionality of the search (Wiktor et al., 2021). In such a picture, the filament itself provides a large target, allowing donor DNA to encounter the filament without requiring the filament to actively scan the entire chromosome. However, our simulations indicate that rigidity alone may not be sufficient if filament expansion along the cell axis is diffusion-limited. When RecN motors were added, the filament expanded more rapidly and traversed the cell more effectively, allowing search to complete soon after the filament reached the donor-containing region. Thus, RecN-driven motion can complement the dimensionality-reduction mechanism by actively establishing the extended filament configuration required for efficient search.

The same principle was also observed in the spherical-cell geometry. In this case, the cell lacks a long axis that could naturally guide pole-to-pole motion, yet diffusion alone still failed to support efficient cell-volume exploration. RecN-driven activity improved search by helping the filament expand and reposition within the available space. This suggests that the main role of RecN is not simply to drive motion along the long axis of rod-shaped bacteria, but more broadly to overcome the slow and inefficient spatial exploration expected from passive filament dynamics.

These results provide a possible explanation for why RecN is broadly conserved in bacterial DSB repair systems (Rocha et al., 2005). Bacterial species differ widely in cell shape, genome size, chromosome organization, and DNA repair machinery, but all face the same physical challenge: a homologous donor sequence may be spatially distant from the break site and must be found on a biologically relevant timescale. RecN-like motor activity offers a general

solution to this challenge by converting local ATP-dependent activity into large-scale motion of the RecA-DNA search complex. Therefore, rather than acting only as a repair scaffold, RecN may provide a conserved physical mechanism for making bacterial homology search spatially efficient.

3.8 Limitations and outlook

While the present model captures a minimal physical mechanism sufficient to explain RecN-driven filament motion during homology search, several simplifying assumptions define the scope of its interpretation. First, the mapping between simulation and biological timescales was based on DNA diffusion behavior and MSD curve fitting (Weber et al., 2010). This provides a useful first-order calibration, but chromosomal locus motion in-vivo is influenced by many factors such as chromosome organization, nucleoid-associated proteins, and active cellular processes. Furthermore, there can also be inhomogeneities in diffusion of different loci depending on their position along the genome and in 3D, as well over time, which I didn't take into account. Therefore, the inferred simulation-to-real-time conversion should be treated as an approximate correspondence rather than an exact physical timescale.

A second limitation is that the physical properties of the RecA filament were treated as static. In-vivo, RecA filaments are dynamic structures whose length, stiffness, local conformation, and interaction behavior can change due to ATP hydrolysis, monomer turnover, accessory proteins, and interactions with DNA. By keeping these properties fixed, the model allowed us to isolate how defined mechanical parameters influence translocation. However, it does not fully capture dynamic filament remodeling, which may slow, interrupt, or redirect motion during the actual search process.

A further simplification concerns the representation of chromosomal DNA. In the simulations, DNA was modeled as a confined polymer with random-walk-like organization. This captures generic polymer behavior and allows the mechanical effects of RecN-driven motion to be isolated, but it does not reproduce the higher-order organization of bacterial chromosomes. In cells, chromosome structure is shaped by supercoiling, nucleoid-associated proteins, replication state, macrodomains, and locus-specific positioning. These features may influence both the accessibility of homologous donor sequences and the routes available for RecA filament motion.

The motor model itself should also be viewed as a minimal realization rather than a complete molecular description of RecN. The simulations assume a particular RecN polarity, transient DNA binding and release, and association with the RecA-DNA complex. These assumptions are biologically motivated, but the exact molecular geometry of RecN action remains unresolved. Therefore, the model establishes that an ATP-dependent RecN-like motor is sufficient to generate the observed type of filament motion, but it does not exclude other microscopic implementations that could produce similar large-scale behavior.

Future work should therefore focus on connecting this physical model more directly to molecular and cellular measurements. Experimentally, it will be important to determine whether RecN binds RecA filaments in a defined orientation, whether RecN activity produces directional DNA displacement, and how RecN association changes during filament translocation. In-vitro measurements of RecN force generation, DNA binding lifetime, and RecA-dependent ATPase activity would help constrain the motor parameters used in the model. On the modeling side, future simulations could incorporate dynamic RecA filament remodeling, more realistic chromosome organization, explicit partial-homology sampling, and species-specific cell geometries. Such extensions would help determine how robust the proposed mechanism remains under more realistic biological conditions. More broadly, this system may also provide a useful model for SMC biology by linking ATP-dependent conformational changes in an SMC-like protein to force generation, DNA displacement, and large-scale motion of a protein-DNA complex.

Overall, this work provides a mechanistic framework for understanding how RecN can transform homology search from a local diffusive process into an active, cell-scale search strategy. Despite its simplifications, the model identifies a minimal set of physical ingredients, ATP-dependent RecN motor activity, RecA filament stiffness, appropriate DSB-end geometry, and weak RecA-DNA interaction, that together are sufficient to support efficient RecA filament translocation during bacterial DSB repair.

4. Materials and Methods

4.1 Model overview: Coarse-grained model for *C. crescentus* cell

We developed a coarse-grained polymer simulation model to investigate the physical mechanism underlying RecN-mediated RecA filament directional translocation during homology search. In this framework, the RecA - ssDNA nucleoprotein filament and the target double-stranded DNA (dsDNA) are represented as simplified polymer-like structures that retain the essential mechanical and topological features governing their interaction. The model focuses on the minimal set of physical ingredients required to capture filament motion, including bending rigidity, excluded volume interactions, and transient filament–DNA binding.

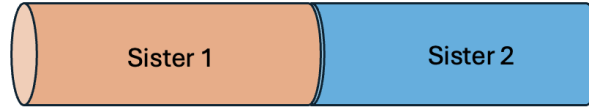
This framework enables systematic investigation of how mechanical properties and geometric constraints influence filament dynamics and search behavior. By abstracting away atomistic and sequence-specific biochemical details, the model isolates the mechanical principles that regulate search efficiency and directional translocations. Parameter choices were guided by intracellular length scales typical of *Caulobacter crescentus*, ensuring that the simulated dynamics remain within a physiologically relevant regime.

4.1.1 Representation of DNA

The double-stranded DNA (dsDNA) was modeled as a coarse-grained polymer chain in which each bead represents approximately 10 nm of DNA, corresponding to about 30 base pairs. Adjacent beads were connected by simple harmonic bonds to preserve chain connectivity while allowing thermal fluctuations. At this coarse-grained level, the DNA was treated as effectively flexible and no explicit bending potential was applied. This approximation was intended to represent the strong reduction in large-scale rigidity expected for bacterial chromosomal DNA. In-vivo, binding of nucleoid-associated proteins introduces bends, kinks, and local structural heterogeneity (Verma et al., 2019). Excluded-volume interactions were imposed between non-bonded beads to prevent unphysical overlap.

The genome of *Caulobacter crescentus* consists of approximately 4.04×10^6 base pairs (https://www.ncbi.nlm.nih.gov/nuccore/NC_011916.1). At the coarse-grained resolution used in this study (~30 base pairs per bead), a full chromosome would correspond to on the order

of 10^5 beads (134764 monomers), which is computationally feasible for the timescales of interest.



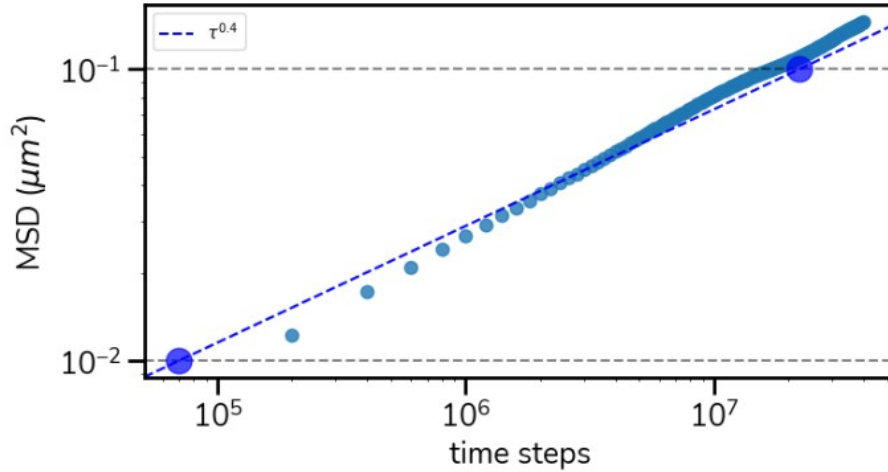
4.1.2 DNA dynamics and time scale mapping

The dynamics of the system were simulated using Langevin dynamics in the overdamped regime, appropriate for intracellular environments where viscous forces dominate over inertial effects. The motion of each bead was governed by deterministic forces arising from bonded and non-bonded interactions, along with stochastic thermal forces representing collisions with the surrounding medium. A friction coefficient $\gamma = 1$ (in simulation units) was used, and the stochastic forces were chosen to satisfy the fluctuation-dissipation theorem, ensuring proper thermal equilibration at a constant temperature.

To relate simulation time to physical time, we calibrated the dynamics of the DNA in our model using experimentally measured diffusion of chromosomal loci in bacterial cells. Specifically, we compared the mean squared displacement (MSD) of DNA beads in our simulations with published MSD measurements of DNA loci reported by (Weber et al., 2010). In our simulations, each bead represents a DNA segment of approximately 10 nm, which sets the length scale for displacement. The MSD curve obtained from the simulations was aligned with the experimental MSD curve by matching displacement magnitudes over comparable spatial scales. Using this correspondence, we identified the number of simulation time steps required to reproduce the experimentally observed displacement over a given time interval.

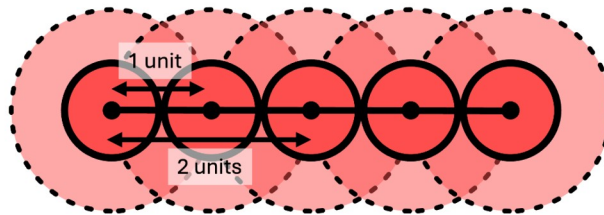
Based on this mapping, we estimated that approximately 4.7×10^5 simulation time steps correspond to ~ 1 s of DNA diffusion in-vivo. This calibration provides a direct conversion between simulation time and physical time, allowing quantitative interpretation of dynamical processes in the model.

For the *E. coli* model the similar mapping yields 5.3×10^5 simulation steps corresponding to ~ 1 s of DNA diffusion in-vivo. Similarly for artificial spherical *C. crescentus* cell 3.8×10^5 simulation steps corresponds to ~ 1 s.



4.1.3 Representation of RecA filament

The RecA - ssDNA nucleoprotein filament was modeled as a coarse-grained polymer chain composed of beads of size 20 nm, corresponding to a larger structural unit compared to the dsDNA representation. Adjacent beads were connected by simple harmonic bonds, same as for DNA, to maintain filament integrity. However, the effective size and spacing of RecA beads were chosen such that adjacent beads overlap in their excluded-volume interactions, as illustrated below. This prevents unphysical passage of DNA segments through the filament and mimics the steric integrity of the nucleoprotein complex. Although the coarse-grained bead size of the filament is larger than that of dsDNA, we chose interaction distances so that DNA cannot pass through the RecA filament.

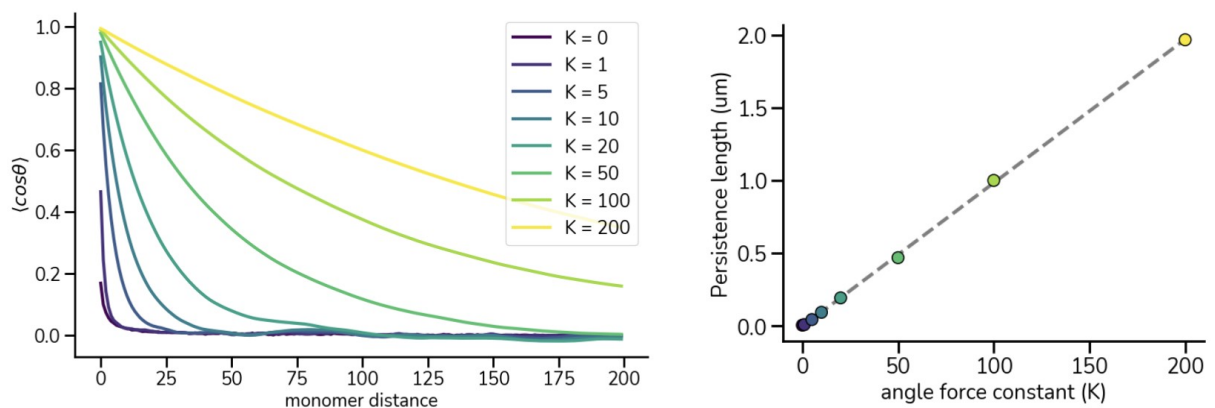


In most of the simulations the length of the filament is fixed at 100 monomers / 1 μm , which is the average length of RecA filaments observed in *C. crescentus* (Chimthanawala et al., 2022). For any given simulation, the filament length is kept fixed throughout the whole trajectories.

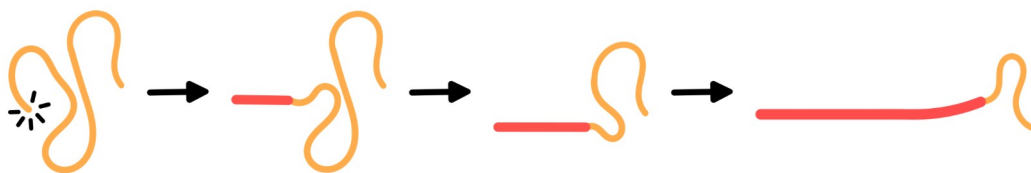
The RecA filament was treated as a polar structure with two distinct ends, referred to throughout the simulations as the tip/head and the tail. This polarity was assigned along the contour of the filament and used consistently to define the orientation of RecN conformational cycles, DNA-end attachment configurations, and the direction of filament motion. For all

analyses, the tip/head was defined as the forward-facing end of the filament, while the tail corresponded to the opposite end. Positive translocation of the filament was therefore defined as motion in the direction of the tip.

In contrast to dsDNA, the RecA filament was assigned higher stiffness through a simple harmonic angular potential between consecutive bonds. This reflects the known mechanical properties of RecA filaments, which form extended and relatively rigid nucleoprotein filaments. The stiffness of the filament was characterized in terms of its persistence length, defined as the characteristic length scale over which directional correlations along the filament are maintained. The persistence length was estimated from simulations by computing the decay of tangent–tangent correlations along the filament contour (figure below, left). Specifically, the correlation between bond vectors separated by a contour distance s was evaluated and fitted to an exponential decay, $\langle t(0) \cdot t(s) \rangle \sim e^{-s/L_p}$, where L_p is the persistence length (figure below, right). Interestingly, the persistence length is directly correlated with the force constant of the harmonic angle potential, and persistence length of 1 μm corresponds to the force constant value of 100.



In every simulation, RecA filament is built i.e. from flexible to stiff, from tip to tail of the filament. To represent the active resection ongoing by the resection machinery. Therefore in my simulation also RecA filament stiffness is introduced segment by segment over the span of $\sim 2\text{kb}$ under $\sim 1\text{min}$, generating 1 μm long filament. For simplicity of our model then we assume the resection stops and filament stays at that length.

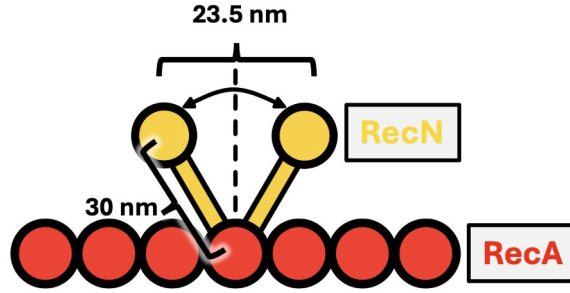


4.1.4 RecA filament - DNA ends attachment geometries

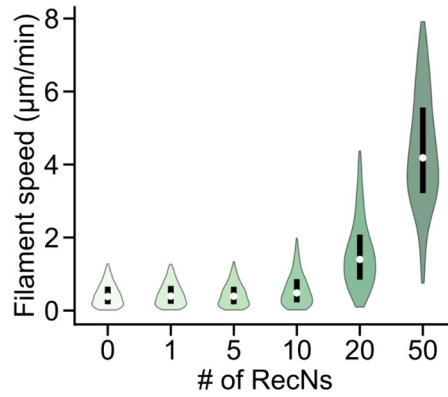
Following the generation of a double-strand break (DSB), two DNA ends are produced. Experimental observations typically reveal the formation of a single RecA filament, suggesting that the two DNA ends remain associated during homology search. In the model, this constraint was enforced by maintaining both DNA ends in association with the filament throughout the simulation. Under this constraint, two possible attachment geometries were considered, as illustrated in Fig. 2.9A. In the first configuration, both DNA ends were attached to the same end of the filament, corresponding to attachment at the tail. In the second configuration, the two DNA ends were attached to opposite ends of the filament, with one end at the tip and the other at the tail. These two geometries are called Pendant and Bridge respectively, in our simulations. These configurations represent the minimal topologically distinct arrangements compatible with a single filament and were used to investigate how end geometry influences filament dynamics and directional motion.

4.1.5 Representation of RecNs

RecN was modeled as a single coarse-grained monomer bound to the RecA filament, representing an elongated protein associated with the nucleoprotein complex. The RecN monomer was attached to the filament through a simple harmonic bond of equilibrium length 3 units or 30 nm (with same strength as DNA), allowing it to remain tethered to the filament while retaining orientational freedom, illustrated below. To capture its conformational flexibility, RecN was allowed to adopt two discrete angular configurations relative to the local axis of the RecA filament, oriented approximately 22.5° to either the left or the right of the normal to the filament, as shown below. The distance covered in these 2 configurations is ~ 2.3 units or 23 nm, which is comparable to the arm length of the RecN subunit. These two states are called as extended and bent in the main text to emphasise on the directionality of the force generated by the RecNs. Broadly, these two states represent simplified conformational states associated with RecN-mediated DNA motor activity and were intended to mimic the structural transitions commonly observed in SMC-like ATPase proteins during DNA engagement and motor-like action.



The numbers of RecNs were kept fixed at 20 for all of the simulations, unless otherwise stated. It was calibrated based on the filament speed with respect to the number of RecNs, as plotted below. At 20 RecNs the filament speed and number of traversals were comparable to the experimentally observed motion (Chimthanawala et al., 2022).



4.1.6 RecN motor cycle and update rules

In addition to the orientational change, RecN was also allowed to reversibly bind to dsDNA as mentioned in the main text. DNA binding and unbinding are coupled to the state of the RecN monomers. In extended configuration it can bind to the nearest DNA monomer within a capture radius of 15 nm from the RecN. Then we assume that it leads to a change in its configuration to bent, pulling the DNA in one direction. Followed by the DNA release and motor reset. So overall there are total 4 ordered functional steps in the RecN motor cycle:

1. Extended configuration, DNA free
2. Extended configuration, DNA bound
3. Bent configuration, DNA bound
4. Bent configuration, DNA free

After the 4th step the motor resets the configuration to the 1st step.

To implement the transition between these 4 steps, I update the configuration of 20% (*update_frequency*) of all RecNs (4 out of 20 in the base simulation) after each 100 timesteps (*update_steps*) till the simulation ends, or otherwise mentioned. It leads to the average number of steps per RecN cycle to be ~2000, as calculated below:

update_frequency: percentage of recNs randomly chooses to update configuration.
update_steps: number of simulation steps after which update is performed.

$$\text{Average steps per RecN cycle} = 4 * \frac{\text{update_steps}}{\text{update_frequency}}$$

For our case, only 20% of the RecNs are updated stochastically after every 100 time steps, then:

$$\begin{aligned} \text{update_frequency} &= 0.2 \\ \text{update_steps} &= 100, \text{ and} \\ \text{Average steps per RecN cycle} &= 4 * 100 / 0.2 = 2000 \text{ steps} \end{aligned}$$

Therefore in 1 sec there are, on average, approximately 23.5 (47000 / 2000) cycles performed.

The above mentioned RecN update steps and frequency (100, 0.2 respectively) were calibrated based on the filament speed and traversal length (which is an indicator of filament stability). Different combinations of the update frequency and steps were tested, and update steps of 100 and frequency of 0.2 produced the motion similar to the experimentally observed traversals. Therefore it was kept fixed for all of the simulations with RecN.

4.1.7 RecN energetics and polarity of motion

Next we also quantified the energy consumed by each RecN in a complete cycle, as calculated below.

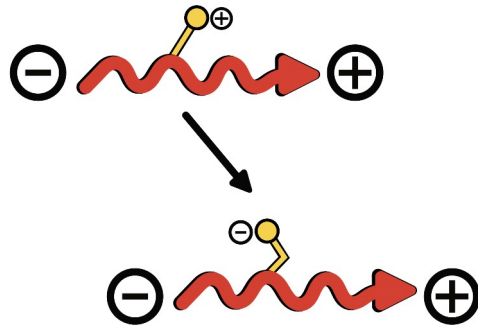
$$\begin{aligned} U &= \frac{1}{2} * k * x^2 \\ &= \frac{1}{2} * 5 * (2.3)^2 \\ &= 13.2 \text{ kT} \sim 1/2 \text{ ATP hydrolysis} \end{aligned}$$

Here, (k = 5) is the harmonic force constant and (x = 2.3) is the displacement between the RecN coordinates in the two conformational states. This gives an energy of ~ 13.2 kT. Thus, the energy consumed per RecN cycle is estimated to be ~13.2 kT, corresponding to roughly half of the free energy released by ATP hydrolysis at room temperature.

Using this energy estimate, the average force generated by each RecN molecule during the force-generating step was approximated as $F_{\text{avg}} = U/x$. Substituting the values above gives $F_{\text{avg}} = 13.2 / 2.3 = \sim 5.7$ kT per simulation length unit. Since 1 kT per simulation length unit corresponds to approximately 0.41 pN in our simulations (see Materials and Methods 4.4), the average force generated during each force-generating step is approximately 2.4 pN.

Note of RecN polarity of motion:

We assume that the polarity of the RecN orientation cycles, where it binds the DNA when facing the tip of the filament (extended configuration) and pulls it towards the tail of the filament (bent configuration) is governed by the polarity of the RecA filament itself. RecA filaments are right handed helix, and their two ends are distinct. It is also known that RecN binding to RecA filaments are very species specific, implying presence of a distinct binding site. So we assume that in the extended configuration RecN faces towards the tip of the filament (+ end in the figure below) and in the bent one it faces towards the tail of the filament (- end of the filament). Such inherent polarity of binding allows all of the RecNs to produce motion in the same direction as that of the tip of the filament, moving it in forward direction, as we observe in the simulations.



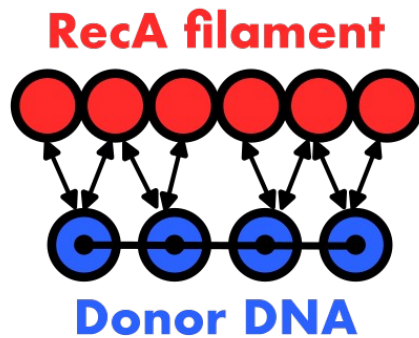
4.1.8 Homology recognition via filament-donor attraction

To model homology recognition in a coarse-grained manner, we introduced a selective attractive interaction between the RecA filament and the donor DNA segment on the sister chromosome. This interaction was designed to reflect the known structural mismatch between RecA-bound ssDNA and B-form dsDNA, wherein RecA extends ssDNA to approximately 1.5 times the contour length of dsDNA.

Experimentally, a RecA filament of length $\sim 1 \mu\text{m}$ would correspond to a DNA segment of approximately 2 kb. Consistent with this ratio, the RecA filament in our simulations was

represented by 100 monomers, while the corresponding homologous donor segment consisted of ~67 DNA monomers ($66.7 \times 30 \text{ bp} \approx 2 \text{ kb}$). To account for this length mismatch, donor recognition was implemented using a 3:2 interaction scheme, in which every 3 adjacent RecA monomers were paired with 2 adjacent donor DNA monomers, as illustrated below.

This “W”-like interaction pattern ensures geometric compatibility between the stretched RecA filament and the shorter donor dsDNA segment. The attractive interaction was activated only when the corresponding filament - donor monomer pair came within a capture distance of 20 nm. Beyond this distance, no donor-specific interaction was applied. Once the pair entered the capture range, the interaction was treated as effectively irreversible over the timescale of the simulation, representing stable homology-dependent pairing. This selective and geometrically constrained attraction provides an effective coarse-grained representation of homology recognition without explicitly modeling sequence-level base pairing.



In the simulations, donor-recognition interactions were implemented only for one side of the DSB. It corresponds to the assumption that although the two DNA ends remain physically associated during homology search, only one end forms the functionally active RecA filament responsible for donor engagement. This represents the minimal configuration required to capture the essential features of directional search and translocation.

We further note that if both DNA ends were to form functional RecA filaments while remaining associated, this would primarily be expected to increase the effective search rate by providing multiple interacting interfaces with the donor DNA, rather than qualitatively altering the underlying mechanism of motion. In this scenario, the two filaments would act cooperatively, effectively enhancing sampling of the chromosomal environment.

4.1.9 Filament - DNA attraction

In addition to the donor-specific interaction described above, we introduced a weak non-specific attractive interaction between the RecA filament and dsDNA monomers. In the absence of such an interaction, we found that the stiff RecA filament frequently drifted toward

and remained near the confinement boundary, rather than exploring the interior DNA-rich region of the simulated cell. Such behavior is well known in the bacterial cells. Previous studies have shown that large complexes such as RNA polymerase subunits which do not interact with dsDNA are excluded from the nucleoid (Landgraf et al., 2012; Stracy et al., 2015; Stylianidou et al., 2014). Whereas DNA-binding proteins undergo extensive transient non-specific interactions with chromosomal DNA during target search (Stracy et al., 2021). This non-specific interaction was, therefore, included to represent the effective tendency of the filament to remain associated with the DNA-rich chromosomal environment, without imposing homology-dependent pairing.

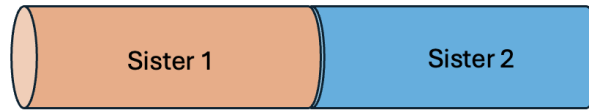
This behavior is also consistent with general polymer-physics expectations in confined systems, where semiflexible or stiff polymers can exhibit effective boundary localization due to confinement and entropic effects (Chauhan et al., 2021; Yethiraj et al., 1994). In-vivo, however, the RecA filament is expected to experience frequent weak contacts with surrounding chromosomal DNA and other nucleoid-associated components, making a mild non-specific filament–DNA attraction a reasonable coarse-grained approximation. Accordingly, a weak short-range attractive interaction was applied between the RecA filament and dsDNA throughout the simulation domain. This attraction was chosen to be sufficiently weak so as not to override donor-specific recognition, but strong enough to maintain the filament within the DNA-rich bulk of the confinement and allow productive exploration of chromosomal DNA.

4.2 Simulation initialization and geometric configurations

All simulations were performed in a cylindrical confinement representing a bacterial cell. The confinement had a length of 500 simulation units and a diameter of 60 simulation units, corresponding to approximately 5 μm in length and 600 nm in diameter. Although the simulations were implemented in HOOMD-blue (J. A. Anderson et al., 2020) using a periodic simulation box, particle motion was restricted to the cylindrical cell volume through the confinement potential described below.

At the start of each simulation, two chromosomes were initialized independently as random-walk polymer conformations in the two halves of the cylindrical cell, representing a dividing cell containing sister chromosomes. The long axis of the cylinder defined the cell axis used for spatial partitioning into the two initial cell halves. One chromosome was designated as intact and served as the donor chromosome, while the other represented the damaged

chromosome. Although the initial conformations were generated in separate cell halves, both chromosomes were allowed to freely diffuse throughout the full cylindrical confinement once the simulation began.



The intact chromosome remained circular, whereas the damaged chromosome contained the RecA filament described above. For each simulation replicate, the spatial positions of both the RecA filament and the donor chromosome were generated independently, resulting in distinct initial search geometries across replicates. The initial filament geometry was therefore not fixed across runs, allowing sampling over a broad range of relative filament - donor configurations. RecN monomers were placed randomly along the filament monomers at the beginning of each simulation. Unless otherwise stated, all RecN monomers were initialized in the extended, DNA-unbound configuration.

To remove artifacts associated with the initial random polymer conformations, the system was first relaxed for several million simulation steps. Following this relaxation phase, I run the simulation for a total of 60 million timesteps corresponding to ~ 22 min. At the beginning of the simulation run, the RecA filament is constructed on the damaged chromosome, followed by donor-specific attraction and RecN motor activity activation. To quantify homology-search efficiency, approximately 100 independent simulation replicates were performed for each condition, except for spherical cells 20-25 were used. For other analyses, approximately 20 independent replicates were used unless otherwise stated. Furthermore, all reported translocation and search behaviors were quantified only after the RecA filament building, unless otherwise stated

4.3 Interaction potentials and simulation parameters

All simulations were performed using HOOMD-blue v3.7.0 under Langevin dynamics. Unless otherwise stated, the integration timestep was set to $dt = 0.025$ (simulation units), the temperature was fixed at $k_B T = 1$, and the friction coefficient was set to $\gamma = 1$.

Langevin dynamics:

The motion of each particle was evolved using Langevin dynamics according to:

$$m_i \frac{d^2 r_i}{dt^2} = F_i - \gamma \frac{dr_i}{dt} + \eta_i(t)$$

where F_i is the total deterministic force on particle i , γ is the friction coefficient, and $\eta_i(t)$ is the stochastic thermal force satisfying the fluctuation - dissipation relation. All quantities are reported in reduced simulation units unless otherwise noted.

Bonded interactions:

Connectivity between adjacent coarse-grained monomers was maintained using a simple harmonic bond potential:

$$U_{bond}(r) = \frac{1}{2} k_b (r - r_0)^2$$

where r is the bond length, r_0 is the equilibrium bond length, and k_b is the bond spring constant.

For dsDNA, RecA filament monomers, and all other bonded pairs unless otherwise specified, the parameters were:

$$r_0 = 1, k_b = 1000$$

For the RecN - RecA bond, the same harmonic form was used with:

$$r_0 = 3, k_b = 1000$$

corresponding to a bond length of 30 nm.

When RecN was bound to DNA, the RecN - DNA interaction was represented using the same harmonic form but with a weaker spring constant:

$$r_0 = 1, k_b = 100$$

Angular interactions:

The dsDNA polymer was modeled as fully flexible, and therefore no explicit angular potential was applied between consecutive DNA bonds.

The bending rigidity of the RecA filament was introduced through a harmonic angular potential:

$$U_{angle}(\theta) = \frac{1}{2} k_\theta (\theta - \theta_0)^2$$

Where θ is the angle formed by three consecutive filament beads, θ_0 is the equilibrium angle and k_θ is the angle spring constant.

For the RecA filament:

$$\theta_0 = 180^\circ$$

In the base simulations corresponding to a persistence length of approximately $1\mu\text{m}$, the angular stiffness was set to:

$$k_\theta = 100$$

For simulations with altered persistence length, the angular stiffness was scaled as:

$$k_\theta = l_p \times 100$$

where l_p denotes the persistence length in micrometers.

RecN conformational switching was implemented separately through discrete state changes between the two prescribed angular configurations relative to the local filament axis, rather than through a continuous angular potential, as described above.

Excluded-volume interactions:

Steric exclusion between non-bonded particles was implemented using the conservative force component of the dissipative particle dynamics (DPD) interaction:

$$U(r) = A \cdot (r_{\text{cut}} - r) - \frac{1}{2} \cdot \frac{A}{r_{\text{cut}}} \cdot (r_{\text{cut}}^2 - r^2).$$

where:

A is the repulsive amplitude

r is the interparticle distance

r_{ij} is the cutoff radius

For most particle types, the parameters were:

$$A = 10, r_{\text{cut}} = 1$$

For RecA filament monomers, a larger excluded-volume cutoff was used:

$$A = 10, r_{\text{cut}} = 2$$

This larger steric radius reflects the larger effective size of the filament beads relative to dsDNA and ensures that adjacent RecA monomers form a nearly continuous steric barrier that prevents unphysical passage of DNA segments through the filament.

Confinement potential

The cylindrical cell geometry was imposed using a Gaussian wall potential implemented through the HOOMD wall force. The wall interaction energy was written as:

$$U(r) = \varepsilon \exp \left(-\frac{1}{2} \left(\frac{r}{\sigma} \right)^2 \right)$$

where:

r is the perpendicular distance from the particle to the wall surface

ε is the wall strength

σ sets the spatial extent of the wall interaction

For all particles these parameters are:

$$\varepsilon = 1, \sigma = 1$$

This potential restricted all particles to the cylindrical confinement representing the bacterial cell while preserving smooth short-range repulsion near the boundary.

Specific and Non-Specific Filament - DNA Interactions:

Both the weak non-specific filament - DNA interaction and the donor-specific filament - DNA interaction were implemented using a tabulated pair potential consisting of a short-range repulsive component and an intermediate-range attractive component. This interaction was constructed to provide smooth, finite-range forces suitable for coarse-grained simulations.

For a pair of interacting particles separated by a distance r , the interaction potential was defined as a piecewise function:

For $r < r_{rep}$:

$$U(r) = E_{rep} \times \left(1 - \left(\frac{r}{r_{rep}} \right)^2 \right)^2$$

For $r_{rep} \leq r \leq r_{att}$:

$$U(r) = -E_{att} \times [1 - ((r - \frac{r_{rep} + r_{att}}{2}) / (\frac{r_{att} - r_{rep}}{2}))^2]^2$$

For $r > r_{att}$:

$$U(r) = 0$$

Here, E_{rep} and E_{attr} denote the strengths of the repulsive and attractive interactions, respectively, while r_{rep} and r_{attr} define the spatial ranges of the repulsive and attractive regions.

The corresponding force was computed as the negative gradient of the potential, yielding:

For $r < r_{rep}$:

$$F(r) = 4 \times \frac{E_{rep}}{r_{rep}} \times (1 - (\frac{r}{r_{rep}})^2) \times \frac{r}{r_{rep}}$$

For $r_{rep} \leq r \leq r_{att}$:

$$F(r) = -4 \times \frac{E_{att}}{\Delta} \times [1 - ((r - \frac{r_{rep} + r_{att}}{2}) / \Delta)^2 \times (r - \frac{r_{rep} + r_{att}}{2}) / \Delta]$$

For $r > r_{att}$:

$$F(r) = 0$$

where

$$\Delta = \frac{r_{att} - r_{rep}}{2}$$

A weak version of the above interaction was applied between all RecA filament beads and dsDNA beads to represent transient, non-specific DNA contacts during homology search. The interaction strength E_{att} was chosen to be small ($E_{att} = 0.3$) compared to the donor-specific interaction, ensuring that the filament remained associated with the DNA-rich environment without forming stable, non-specific attachments.

To model homology recognition, a stronger and geometrically constrained version of the same interaction potential was applied between the RecA filament and the donor DNA segment, as described above. The attractive interaction ($E_{att} = 10$) promoted stable pairing between the filament and the donor DNA, effectively representing homology-dependent recognition at a coarse-grained level.

4.4 Quantification and analysis

Filament velocity and traversal time:

Filament motion was quantified using the mean position of RecA filament monomers along the long axis of the cylindrical cell. Only the axial coordinate was used, as filament motion was predominantly oriented along this direction. The instantaneous speed was computed from the time derivative of this mean axial position. To avoid artifacts from boundary interactions, velocity calculations were restricted to configurations in which the filament remained at least 0.5 μm away from either cell pole. Similarly, the traversal time was defined as the time required for the filament to move between positions located 0.5 μm from opposite cell poles along the long axis. This definition excludes boundary effects and reflects translocation across the effective search region of the cell.

Homology search efficiency:

Homology search efficiency was quantified using the first contact time between the correct segment of RecA filament and the donor DNA. A search event was classified as successful when the filament made its first donor-specific contact at any time during the simulation. For each condition, the search efficiency was defined as the fraction of simulation replicates in which such a first donor contact occurred within 20 min of simulated time. This provided a direct measure of the probability of successful donor encounter within a biologically relevant time window.

Mean Squared Displacement (MSD):

The mean squared displacement (MSD) was calculated in three dimensions for both DNA and the RecA filament as

$$\text{MSD}(\Delta t) = \langle |\mathbf{r}(t + \Delta t) - \mathbf{r}(t)|^2 \rangle$$

where $\mathbf{r}(t)$ denotes the three-dimensional position vector at time t . For the filament, $\mathbf{r}(t)$ corresponds to the mean position of all filament monomers.

MSD values were computed using all pairwise frame combinations to generate an MSD matrix, and the final MSD curve was obtained by averaging over the matrix diagonals corresponding to equal time intervals Δt . This procedure effectively averages over all possible time origins and was used to quantify both DNA and filament mobility. The simulated DNA MSD was further used for time-scale calibration, as described above.

Filament stall force estimation:

The stall force of the filament was estimated from the relationship between filament velocity and externally applied opposing force. For this analysis, a constant force was applied to the filament, and the resulting steady-state velocity of the filament tail was measured along the long axis of the cell.

To express the applied force in physical units, simulation forces were converted to piconewtons using the mapping between thermal energy and length scale in the model. In physical units, $1 k_B T \approx 4.1 \text{ pN}\cdot\text{nm}$ at room temperature. Since one simulation length unit corresponds to 10 nm, a force of $1 k_B T$ per simulation length unit corresponds to approximately

$$4.1 \text{ pN}\cdot\text{nm} / 10 \text{ nm} = 0.41 \text{ pN}.$$

Thus, forces applied in simulation units were multiplied by 0.41 to obtain the corresponding force in pN.

The measured filament tail velocity was then plotted as a function of the opposing force. The stall force was defined as the force at which the fitted velocity extrapolated to zero. To estimate this value, a linear fit was performed using only the first three data points in the force–velocity curve, corresponding to the regime in which the filament continued to move forward under load. Data points at larger forces were excluded from the fit, as they corresponded to forces exceeding the stall force and led to backward filament motion. Using this procedure, the filament stall force was estimated to be approximately 1.03 pN.

DNA span:

The spatial extent of DNA was quantified using the axial span of a selected chromosomal segment. Specifically, a 10 kb region was considered, corresponding to approximately $(10,000 / 30) \approx 333$ beads in the coarse-grained representation. For each frame, the DNA span was defined as the difference between the maximum and minimum axial (z-coordinate) positions of the beads belonging to this segment:

$$\text{Span} = \max(z_i) - \min(z_i)$$

where z_i denotes the axial position of bead i within the selected DNA segment. This measure provides an estimate of the longitudinal extension of DNA along the cell axis.

Filament traversal length:

The traversal length was defined as the axial displacement of the filament before reversal of its direction of motion. It was measured using the mean position of the filament along the long

axis of the cell over the full trajectory. For each traversal event, the displacement between the start of a persistent forward motion segment and the point at which the filament reversed direction was recorded. A direction reversal was defined as a 180° change in the direction of axial filament motion. Thus, traversal length corresponds to the distance traveled by the filament along the cell axis before undergoing a complete reversal in its direction of movement. As for the velocity and traversal time analyses, regions within 0.5 µm of either cell pole were excluded to avoid artifacts arising from boundary interactions. Traversal lengths were therefore measured only within the effective central search region of the cell.

Filament Longitudinal and Radial distribution:

The spatial distribution of the filament within the cylindrical confinement was quantified using the positions of all RecA filament monomers over time. The longitudinal distribution was calculated from the axial coordinates of filament monomers along the long axis of the cell, providing the probability distribution of filament occupancy along the cell length.

The radial distribution was calculated from the distance of each filament monomer from the central axis of the cylinder, defined as

$$r = \sqrt{(x^2 + y^2)}$$

where x and y are the coordinates perpendicular to the long axis of the cell. This measure was used to quantify the tendency of the filament to localize either near the center of the confinement or toward the cylindrical boundary.

In addition to the full-filament distribution, these distributions were also computed separately for the first 10, middle 10, and last 10 filament monomers to compare the spatial behavior of different filament regions. Unless otherwise stated, all distributions were obtained by pooling monomer positions over time and across simulation replicates.

Software used:

All simulations were performed using HOOMD-blue v3.7.0 (J. A. Anderson et al., 2020). Filament building and RecN conformational switching were implemented via Hood Custom Actions. Custom simulation routines, including donor-recognition interactions, and trajectory analysis, were implemented using Python scripts. Post-processing and quantitative analyses were carried out using standard Python-based tools, including NumPy, SciPy, and Matplotlib, unless otherwise stated. All of the simulation snapshots and videos were made using either VMD and/or Matplotlib.

Statistical Analysis:

Unless otherwise stated, all reported quantities were computed independently for each simulation replicate and then averaged across runs. For homology-search efficiency measurements, approximately 100 independent replicates were used per condition, whereas most other analyses were performed using approximately 20 independent replicates. For artificial *C. crescentus* spherical cells only 15-25 independent replicates were used per condition for both homology search efficiency measurements and further analysis. Distribution-based quantities were obtained by pooling data over time and across replicates.

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6. Abbreviations

DNA	Deoxyribonucleic Acid
DSB	Double Strand Break
dsDNA	Double Stranded DNA
ssDNA	Single Stranded DNA
SMC	Structural Maintenance of Chromosomes
HDR	Homology-directed repair
ROS	Reactive Oxygen Species
SSB	Single Strand Binding
UV	Ultra Violet
ICL	Interstrand crosslinking
bp	basepair
Mb	Megabasepair
kb	kilobasepair
nm	nanometer
μm	micrometer
NHEJ	Non-Homologous End Joining
SSA	Single-Strand Annealing
MMEJ	Microhomology-Mediated End Joining
BER	Base excision repair
D-loop	Displacement loop
RPA	Replication protein A
Dmc1	DNA meiotic recombinase 1
ms	millisecond
Gb	Gigabase
ATP	Adenosine triphosphate
Chi	Crossover hotspot instigator

HR	Homologous Recombination
pN	Piconewton
FDA	Filament donor attraction
ADP	Adenosine diphosphate