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# The excluded DNA strand is SEW important for hexameric helicase unwinding

Sean M. Carney [a], Michael A. Trakselis [a,b,*]

[a] Molecular Biophysics and Structural Biology Program, University of Pittsburgh, Pittsburgh, PA 15260, United States
[b] Department of Chemistry and Biochemistry, Baylor University Waco, TX 76798, United States

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ABSTRACT

Helicases are proposed to unwind dsDNA primarily by translocating on one strand to sterically exclude and separate the two strands. Hexameric helicases in particular have been shown to encircle one strand while physically excluding the other strand. In this article, we will detail experimental methods used to validate specific interactions with the excluded strand on the exterior surface of hexameric helicases. Both qualitative and quantitative methods are described to identify an excluded strand interaction, determine the exterior interacting residues, and measure the dynamics of binding. The implications of exterior interactions with the nontranslocating strand are discussed and include forward unwinding stabilization, regulation of the unwinding rate, and DNA damage sensing.



**Contents**



*Abbreviations:* AAA$^+$, ATPases associated with various activities; ARDD, average relative deuterium uptake; BER, base excision repair; BrdU, bromodeoxyuridine; CHiP, chromatin immunoprecipitation; CMG, Cdc45/MCM2-7/GINS; CPD, cyclobutane pyrimidine dimer; DNA, deoxyribonucleic acid; dsDNA, double stranded DNA; *Ec*, *Escherichia coli*; FA, Fanconi anemia; FRET, fluorescence resonance energy transfer; FT, Fourier transform; HDX, hydrogen-deuterium exchange; HR, homologous recombination; IdU, iododeoxyuridine; MCM, minichromosomal maintenance; MMR, mismatch repair; MS, mass spectrometry; MS-MS, tandem mass spectrometry; NER, nucleotide excision repair; NTP, nucleotide triphosphate; SE, steric exclusion; SEW, steric exclusion and wrapping; SF, superfamily; smFRET, single molecule FRET; ssDNA, single stranded DNA; *Sso*, *Sulfolobus solfataricus*; *Ta*, *Thermoplasma acidophilum*; TIRF, total internal reflectance fluorescence; UV, ultraviolet light.

* Corresponding author at: One Bear Place #97365, Waco, TX 76798, United States.
*E-mail address:* michael_trakselis@baylor.edu (M.A. Trakselis).

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## 1. Introduction

The loading, activation, and action of hexameric DNA helicases are tightly regulated to occur during the initiation and elongation phases of DNA replication. Hexameric helicases have generally evolved a toroidal geometry and are structurally classified based on having either RecA folds or within the broader ATPases associated with a variety of cellular activities ($AAA^+$) clade [1–3]. RecA hexameric helicases are within the superfamily (SF) 4 and are of some of the most well studied including: T7 gp4, T4 gp41, bacterial DnaB, and mitochondrial Twinkle. Hexameric $AAA^+$ SF3 helicases have conserved Walker A and B motifs, an arginine finger coming from an adjacent subunit to make up the ATPase site, and a unique motif C and are from DNA viruses that include E1 and SV40 Large T-antigen. The archaeal and eukaryotic SF6 hexameric $AAA^+$ MCM helicases have similar ATPase sites comprised from adjacent subunits but also include additional sensor-1 and sensor-2 motifs that function *in trans* to one another to control ATP hydrolysis [4]. Although both structural and functional motifs differ across the three hexameric DNA helicase SFs [3,4,6], the overall three dimensional structure has evolved to encircle and separate DNA strands processively [5].

The prevailing view is that most hexameric helicases encircle one DNA strand (ssDNA) within their central channel, while physically excluding the complementary strand to the exterior [6]. Although it has been shown that hexameric helicases can accommodate double stranded DNA (dsDNA) within the central channel, dsDNA translocation does not appear to contribute to effective DNA unwinding [7]. The exception may be SV40 Large-T-antigen which is known to encircle dsDNA, however, the added ability to bind and melt origin DNA of this SF3 helicase may have captured this specific conformation prior to conversion of encircling only a single-strand [8,9]. Nevertheless, in all cases, hexameric helicases utilize the energy from nucleoside triphosphate (NTP) hydrolysis to propagate the destabilization of hydrogen bonding within the duplex [10]. Hydrolysis at the conserved Walker A and B motifs combined with the other *cis* and *trans* acting elements engage contacts with the translocating strand and propel the helicase forward in a series of steps.

NTP hydrolysis is proposed to occur in a sequential mechanism around the hexamer [11–13]. The conformation of the hexamer has been shown to exist in multiple states including a flat washer, a cracked-ring, or a split spiral [12,14–18]. The degree of out-of-plane spiraling may correlate with the NTPase associated step size, such that additional contacts of the translocating strand within a spiral ring contribute to greater step sizes [19,20]. Whether the global conformation of the hexamer changes or remains fixed during the course of unwinding or for every step is fascinating aspect of the helicase mechanism that is not yet solved.

Once loaded onto ssDNA, hexameric helicases have specific unwinding polarities, where SF4 helicases translocate 5′–3′ and SF3 (except for E1) and SF6 helicases translocate 3′–5′. Strand separation is stimulated by the presence of a steric block, such as the nontranslocating strand. The steric exclusion (SE) model (Fig. 1) of unwinding has been accepted for decades to explain the unwinding action of not only hexameric helicases, but also monomeric and dimeric helicases. One limitation of the SE model is that it generally ignores any contribution of the excluded strand in the unwinding mechanism. Interactions with the excluded strand have been shown previously with hexameric helicases [21], but it was only recently that their role in the DNA unwinding mechanism has been revealed [22]. Although, it has been reported previously, that the ssDNA is bound in the central channel and not wrapped around the DnaB hexamer [23,24], we would hypothesize that external surface binding of ssDNA is not thermodynamically stable when the primary central binding site is available. Only after encircling the 5′-strand of fork DNA would the excluded 3′-strand be conformationally favored for exterior surface binding. In support, binding of a second ssDNA strand to T7 gp4 and *Ec*DnaB helicases has been measured but with lower affinity [25,26]. Therefore, we have expanded the SE model to include favorable interactions with not only the translocating strand but also the excluded strand and termed this the steric exclusion and wrapping (SEW) model for unwinding (Fig. 1). In this methods review, we will detail experimental techniques used to determine the importance and influence of the excluded strand in the DNA unwinding mechanism.

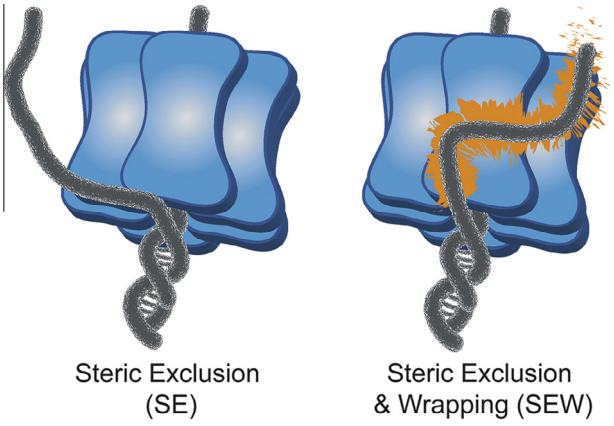


**Fig. 1.** Structural models for hexameric helicase DNA unwinding include steric exclusion (SE) or steric exclusion and wrapping (SEW) where the nontranslocating strand makes contact with the exterior surface (orange).

## 2. Experimental methods for detecting excluded strand contacts

To determine whether exterior interactions of helicases with the nontranslocating DNA strand exist, a variety of qualitative and quantitative biochemical and biophysical experiments can be performed. Both stable and dynamic binding of the nontranslocating strand to the exterior surface may aid in DNA unwinding, and assays are needed to differentiate strands and quantitate specificities. Precise detection of nontranslocating strand binding coupled with mutagenesis can unequivocally determine whether the excluded strand plays any role in DNA unwinding and stabilization for DNA helicases (Table 1).

**Table 1**
Comparison of methods used to validate the Hexameric Helicase SEW Model.

| Method | Experimental advantages |
|---|---|
| DNA footprinting | Identify specific regions and lengths of each strand of DNA protected upon binding to the helicase |
| DNA crosslinking | Captures both transient and stable covalent protein-DNA complexes for analyses of strand specificities and amino acid identification |
| HDX-MS | Global unbiased measurement of DNA binding to the helicase in solution without perturbations |
| smFRET | Determines populations of distance-based DNA conformations and their changes upon wild-type or mutant helicase binding |
| ExPRT analyses | Allows for easy visualization of conformational transitions, binding dynamics, and transition rates of fluorescently labeled DNA strands between two or more experimental conditions |

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