

Force and ATP hydrolysis dependent regulation of RecA nucleoprotein filament by single-stranded DNA binding protein

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Received August 18, 2012; Revised October 24, 2012; Accepted October 27, 2012

ABSTRACT

In *Escherichia coli*, the filament of RecA bound on single-stranded DNA (ssDNA) is essential for recombination and DNA repair. Although ssDNA-binding protein (SSB) plays a complicated role in RecA reactions *in vivo*, much of our understanding of the mechanism is based on RecA binding directly to ssDNA. Here we investigate the role of SSB in the regulation of RecA polymerization on ssDNA, based on the differential force responses of a single S76-nucleotide-long ssDNA associated with RecA and SSB. We find that SSB significantly inhibits the formation of RecA, resulting in inhibition of RecA nucleation. In addition, we find that pre-formed RecA filaments de-polymerize at low force in an ATP hydrolysis- and SSB-dependent manner. At higher forces, re-polymerization takes place, which drops SSB from ssDNA. These findings provide a physical picture of the competition between RecA and SSB under tension on the scale of the entire nucleoprotein RecA army, which have broad biological implications, particularly with regard to competitive molecular binding.

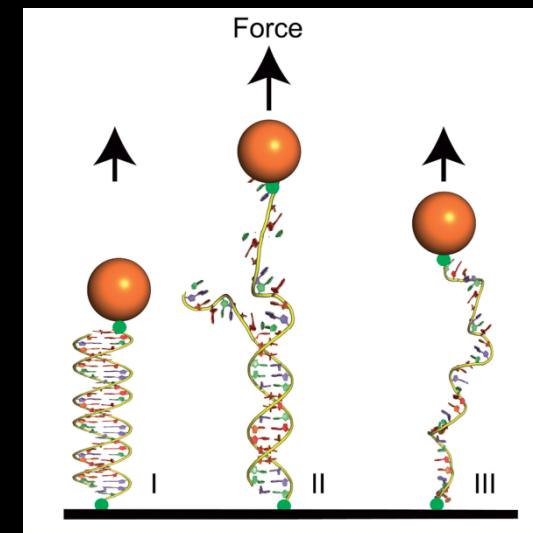
INTRODUCTION

In *Escherichia coli*, the highly cooperative polymerization of RecA on single-stranded DNA (ssDNA) is essential for recombination and DNA repair. The RecA nucleoprotein filament catalyzes recombination-like reactions in the presence of Adenosine Triphosphate (ATP) or its analogs [adenosine triphosphate (ATP)_n] (1,2). The formation of the RecA nucleoprotein filament on ssDNA and its function

kinetically dictate major a slower nucleation step, followed by a faster directional polymerization step. ATP hydrolysis makes the RecA nucleoprotein filament a dynamic structure, the stability of which is determined by polymerization and de-polymerization on ssDNA (3–5). Much of our knowledge of the formation of active RecA nucleoprotein filament is based on bulk biochemical studies (3–6). The recent development of a combination of single-molecule manipulation techniques has begun to contribute to a better understanding of the formation and dynamics of the RecA nucleoprotein filament (6–10). The kinetics of RecA nucleation, directional polymerization, and depolymerization on ssDNA have been measured directly at high spatial and temporal resolution (3,8,10). For example, magnetic and optical tweezers as well as single-molecule fluorescence resonance energy transfer (FRET) have been used to investigate the mechanism of homology search and alignment by the RecA nucleoprotein filament in real time (11–14). However, despite such advances, gaps exist in our understanding of how the formation of RecA nucleoprotein filament is controlled by other nucleoproteins. For example, the *in vivo* relevance for the formation of the RecA nucleoprotein filament as well as for other DNA damage signaling and processing proteins is not ruled *in vitro*, but rather it is “smooth” or “bundled” form of ssDNA-RecA complex (15–18).

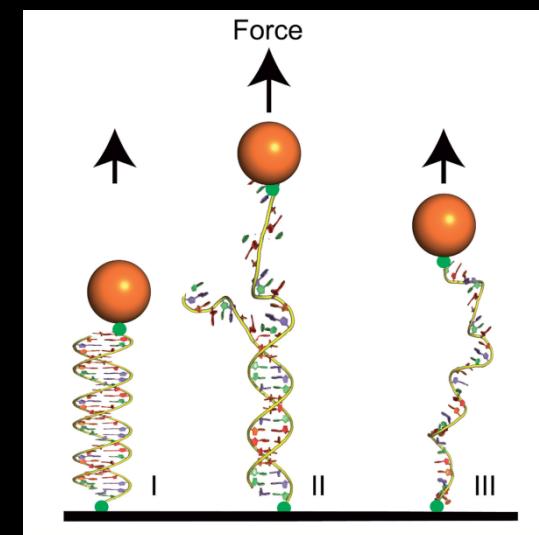
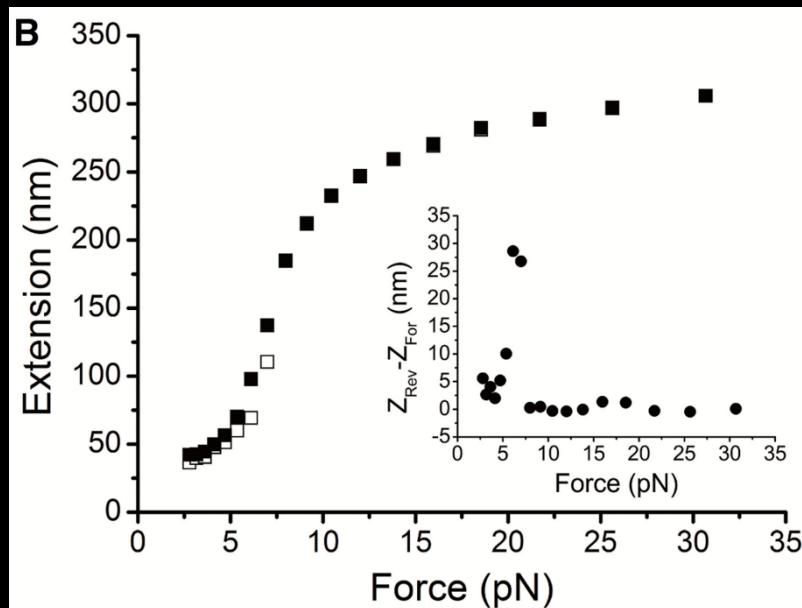
RecA interacts with other nucleoproteins (SSB) to bind also on ssDNA (15). SSB proteins bind to ssDNA non-specifically with multiple modes and high affinity (15). Although the action of SSB on RecA binding to DNA have been extensively studied (3–6), the consequences of competition between RecA and SSB on the formation of RecA nucleoprotein filaments have not been investigated at single-DNA molecule resolution

Force and ATP hydrolysis dependent regulation of RecA filament by SSB

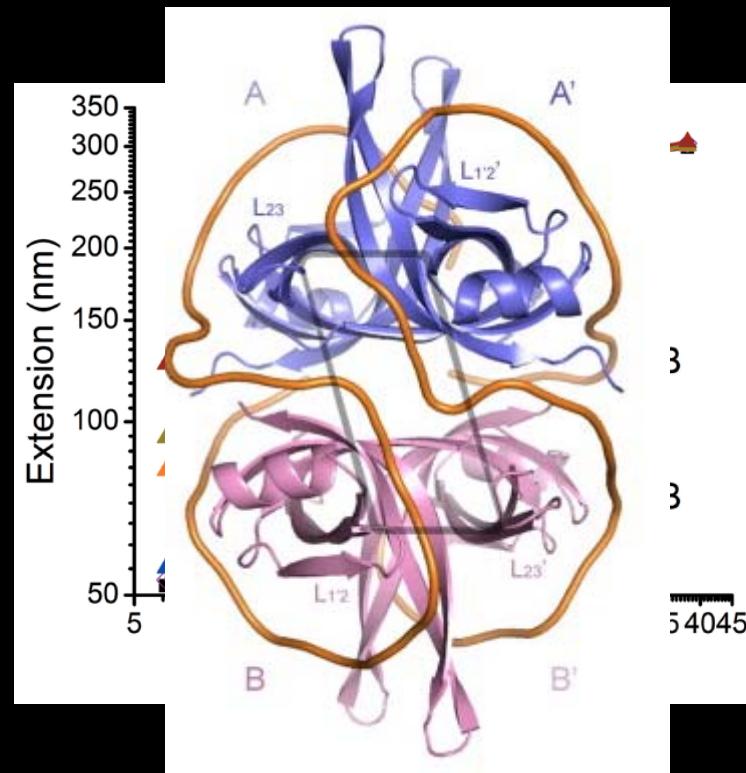
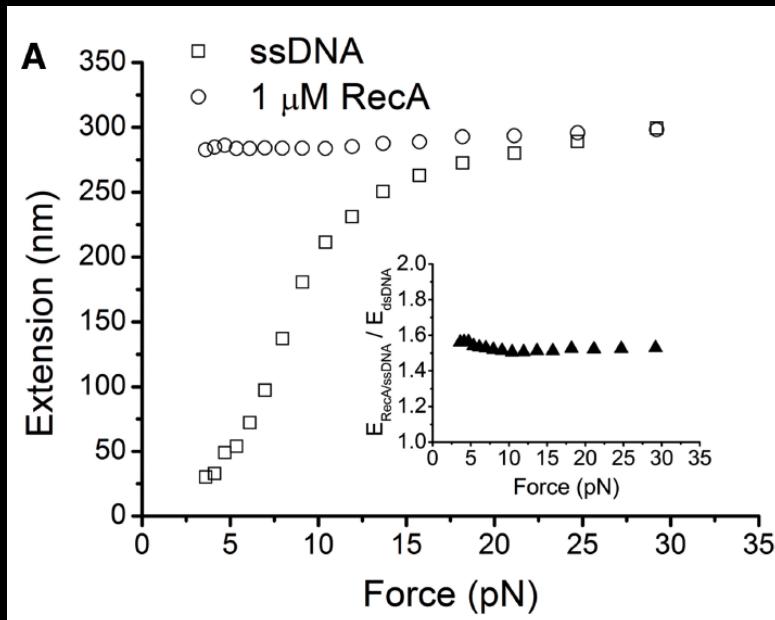


Force-extension curve of ssDNA

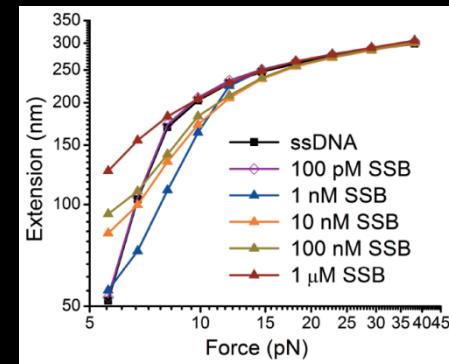
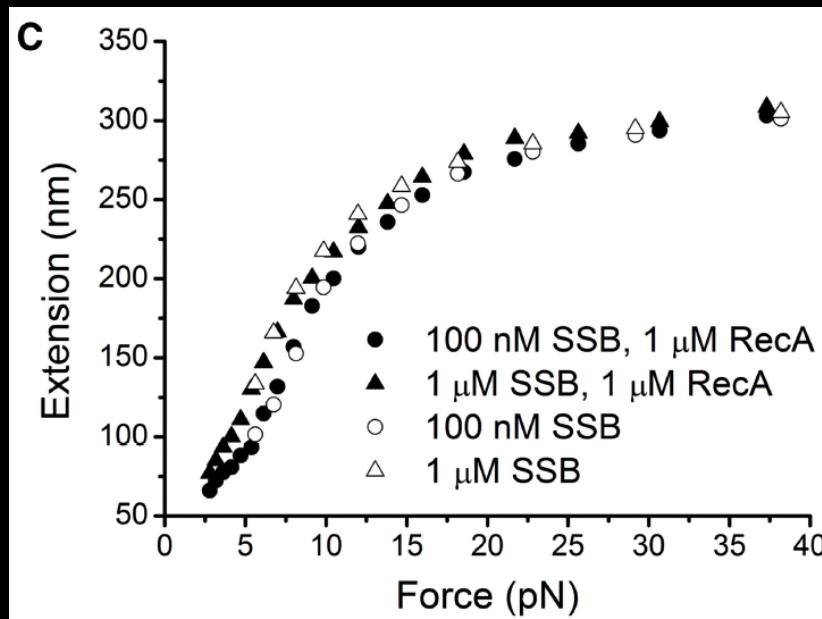
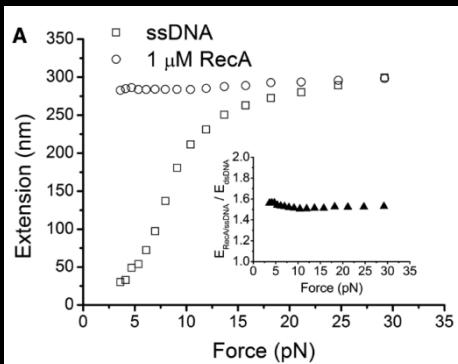
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Force-extension curve of ssDNA-RecA

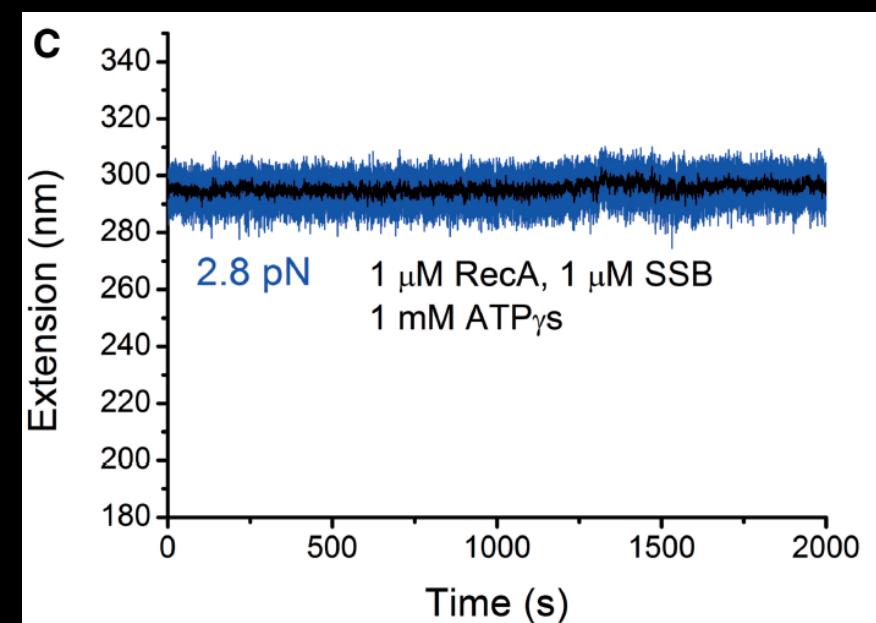
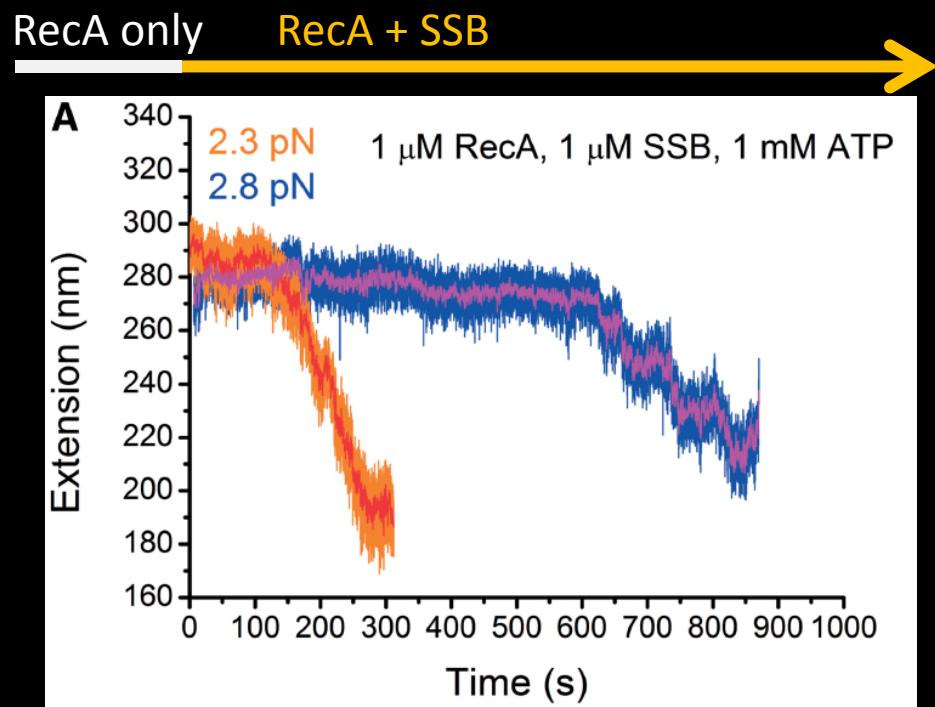


RecA-SSB competition



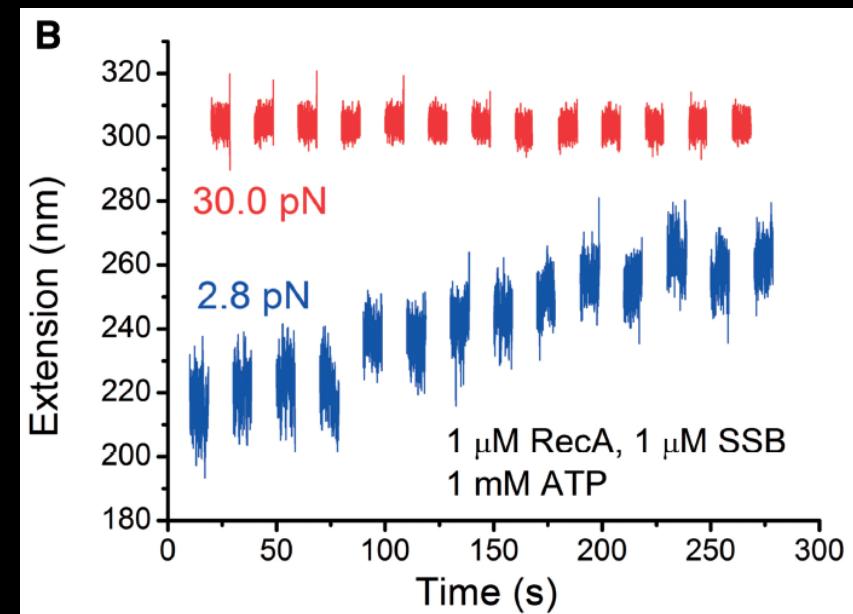
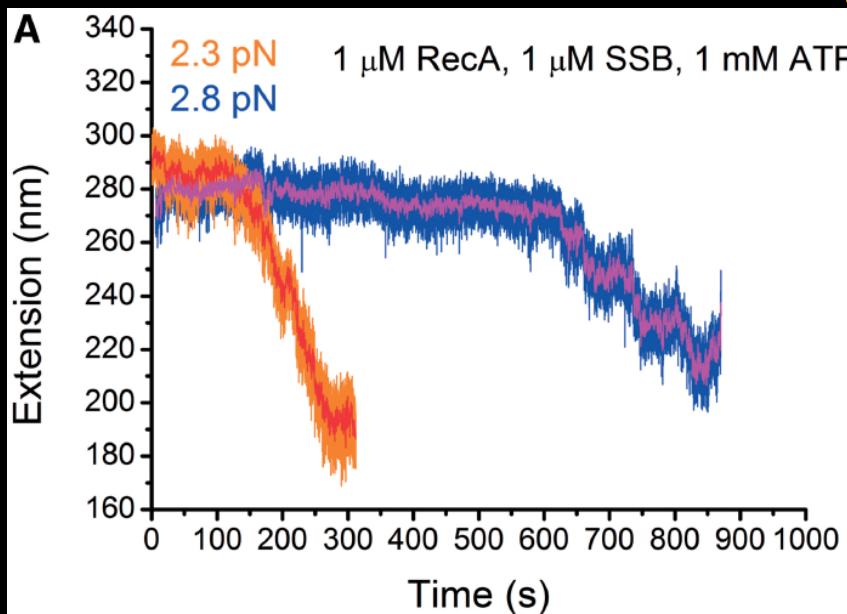
RecA and SSB
introduced at the same time

RecA-SSB competition



RecA-SSB competition

RecA only RecA + SSB



The MODEL

