Single-molecule imaging of DNA pairing by RecA reveals a three-dimensional homology search

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LETTER

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Single-molecule imaging of DNA pairing by RecA reveals a three-dimensional homology search

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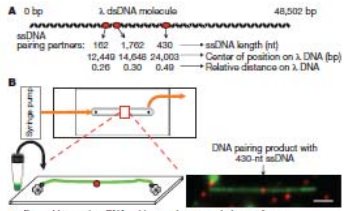
DNA breaks can be repaired with high fidelity by homologous recombination. A ubiquitous protein that is essential for this DNA template-directed repair is RecA[1]. After resection of broken DNA to produce single-stranded DNA (ssDNA), RecA assembles on this ssDNA into a filament with the unique capacity to search and find DNA sequences in double-stranded DNA (dsDNA) that are homologous to the ssDNA. This homology search is vital to recombinational DNA repair, and results in homologous pairing and exchange of DNA strands. Homologous pairing involves DNA sequence-specific target location by the RecA–ssDNA complex. Despite decades of study, the mechanism of this enigmatic search process remains unknown. RecA is a DNA-dependent ATPase, but ATP hydrolysis is not required for DNA pairing and strand exchange[2,3], eliminating active search processes. Using dual optical trapping to manipulate DNA, and single-molecule fluorescence microscopy to image DNA pairing, we demonstrate that both the three-dimensional conformational state of the dsDNA target and the length of the homologous RecA–ssDNA filament have important roles in the homology search. We discovered that as the end-to-end distance of the target dsDNA molecule is increased, constraining the available three-dimensional (3D) conformations of the molecule, the rate of homologous pairing decreases. Conversely, when the length of the ssDNA in the nucleoprotein filament is increased, homology is found faster. We propose a model for the DNA homology search process termed 'intersegmental contact sampling', in which the intrinsic multivalent nature of the RecA nucleoprotein filament is used to search DNA sequence space within 3D domains of DNA, exploiting multiple weak contacts



three different loci of λ DNA (Fig. 1A) was generated by incorporation of 5-(3-aminoallyl) dUTP into ssDNA using polymerase chain reaction (PCR), followed with covalent attachment of ATTO565 (Supplementary Methods). RecA nucleoprotein filaments were assembled on these fluorescent ssDNA substrates in ensemble reactions containing ssDNA-binding protein (SSB) and the non-hydrolysable ATP analogue, ATPγS (5′-O-3′-thiotriphosphate)[4]. ATPγS was used to maintain the filament in its active form, eliminate filament disassembly and prevent dissociation of DNA pairing products[7,10–12]. Using

protein filaments interacting with bacteriophage λ dsDNA in real time by using total internal reflected fluorescence microscopy (TIRFM)[9]. Fully homologous fluorescent ssDNA that was complementary to

proximity (B, d). Homologously paired products were observed in B, c and B, d when DNA was relaxed by stopping flow and then extended by flow for visualization. Scale bars, 2.4 μm.

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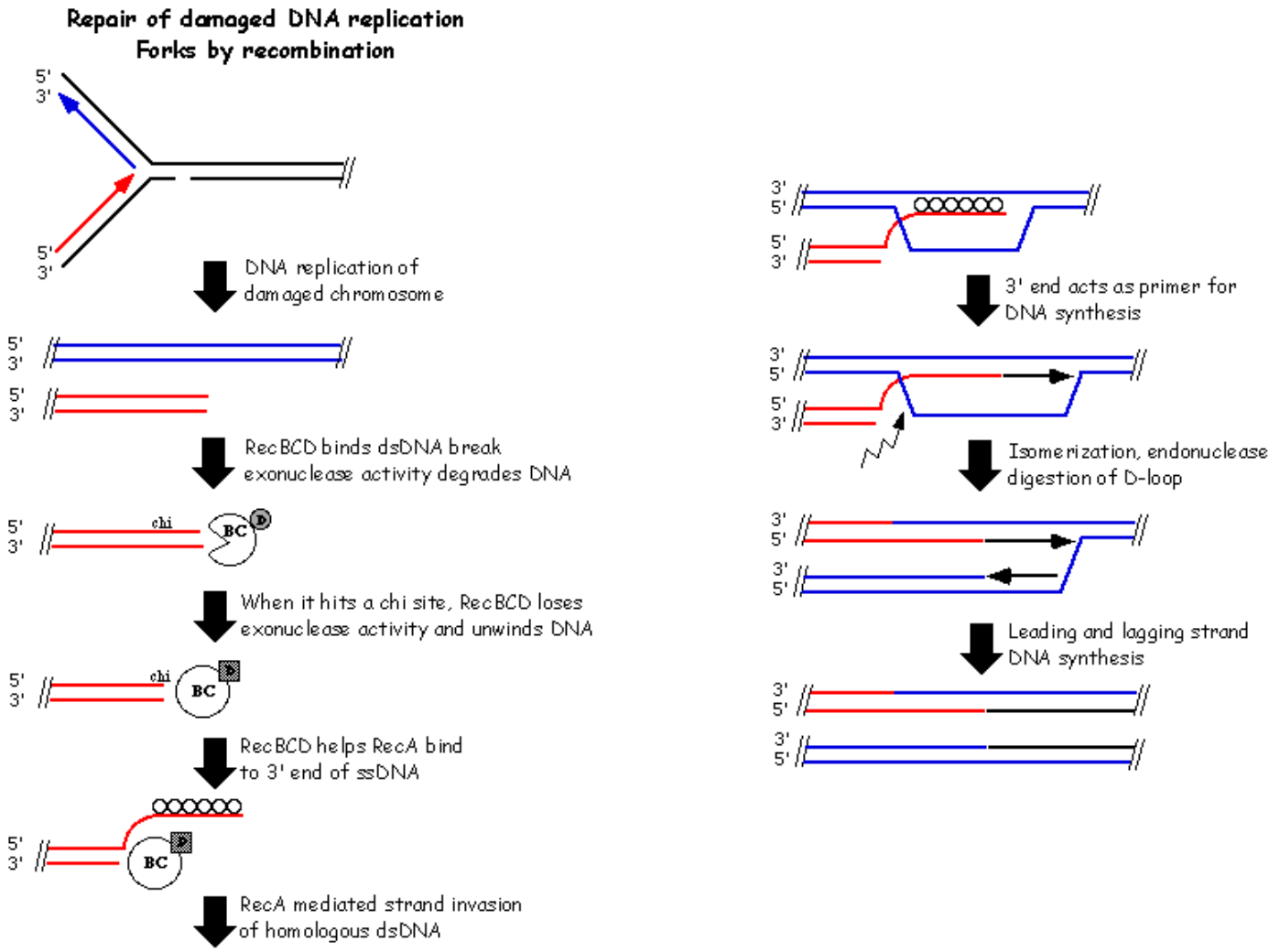
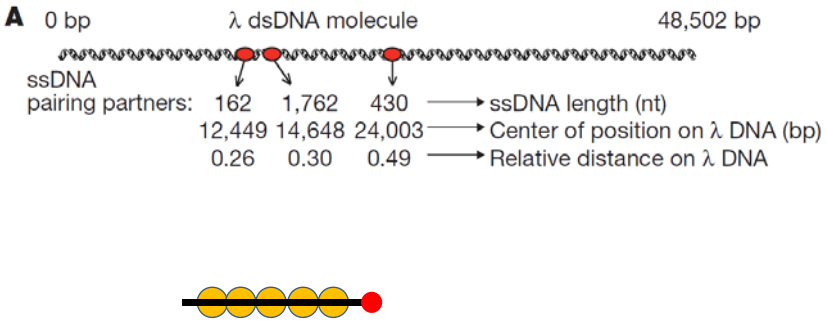
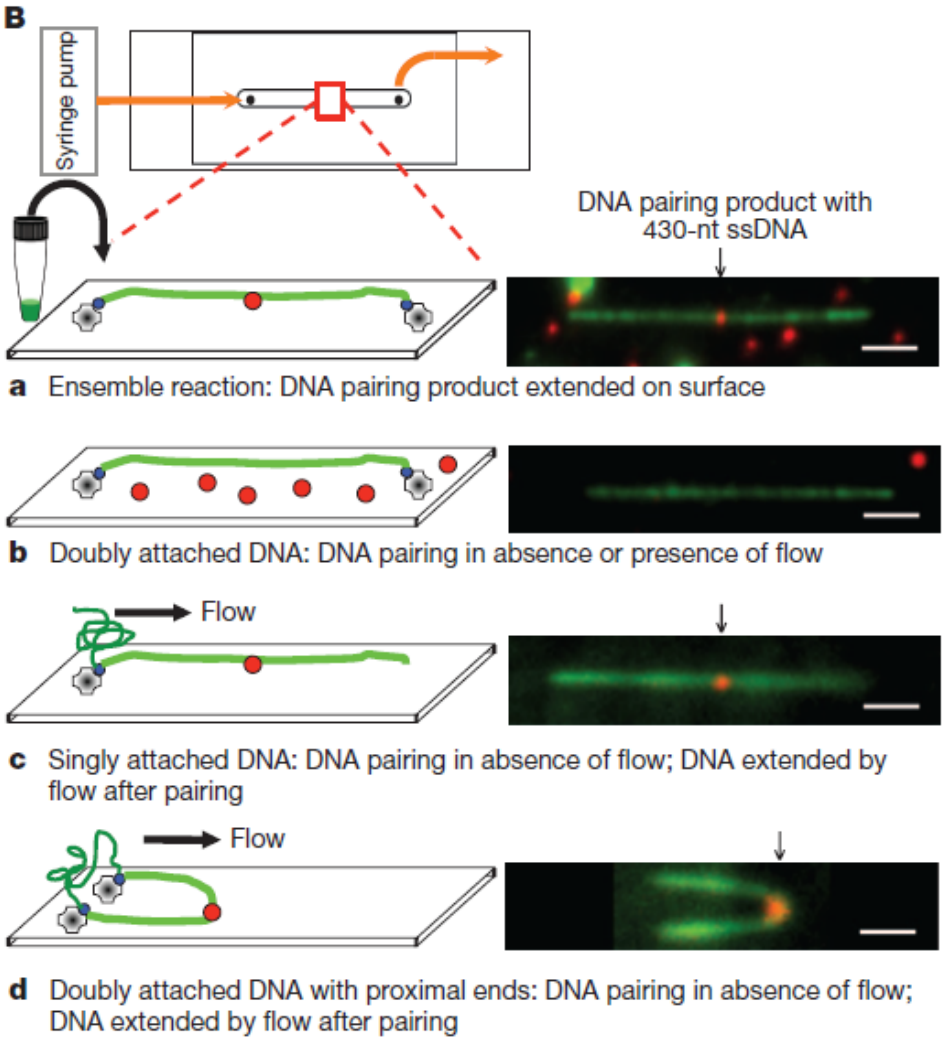
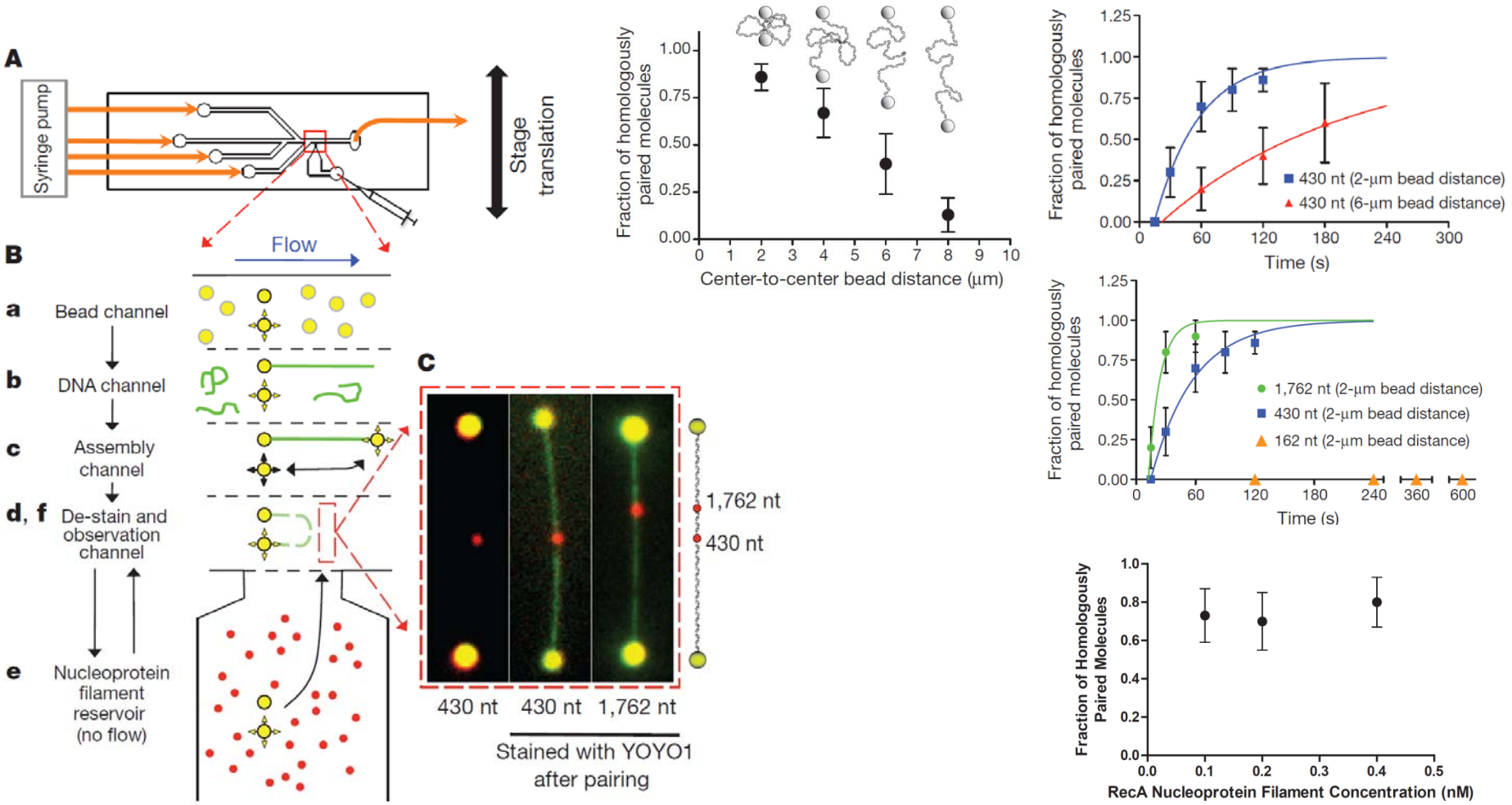
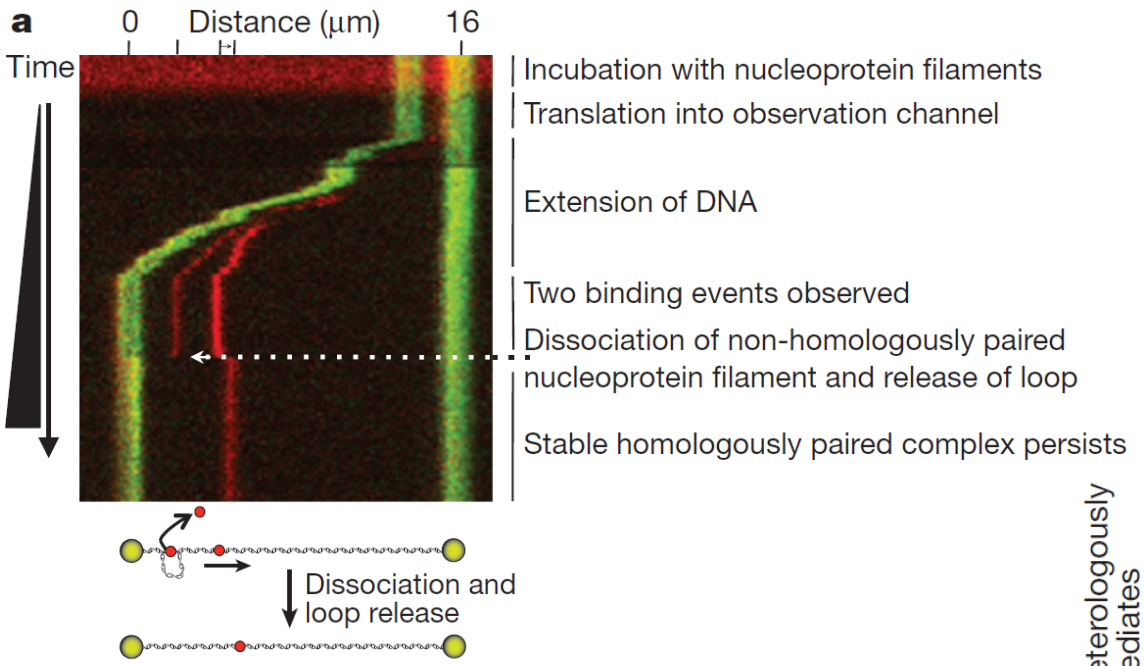
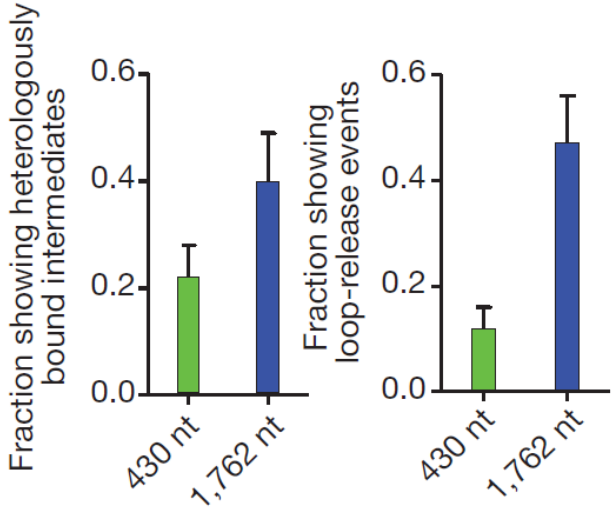

Repair of damaged DNA replication
Forks by recombination
5'
3'
5'
3'
DNA replication of
damaged chromosome
5'
3'
5'
3'
RecBCD binds dsDNA break
exonuclease activity degrades DNA
5'
3'
chi
BC
D
When it hits a chi site, RecBCD loses
exonuclease activity and unwinds DNA
5'
3'
chi
BC
RecBCD helps RecA bind
to 3' end of ssDNA
5'
3'
BC
RecA mediated strand invasion
of homologous dsDNA
3'
5'
5'
3'
3' end acts as primer for
DNA synthesis
3'
5'
5'
3'
Isomerization, endonuclease
digestion of D-loop
3'
5'
3'
5'
Leading and lagging strand
DNA synthesis
3'
5'
3'
5'

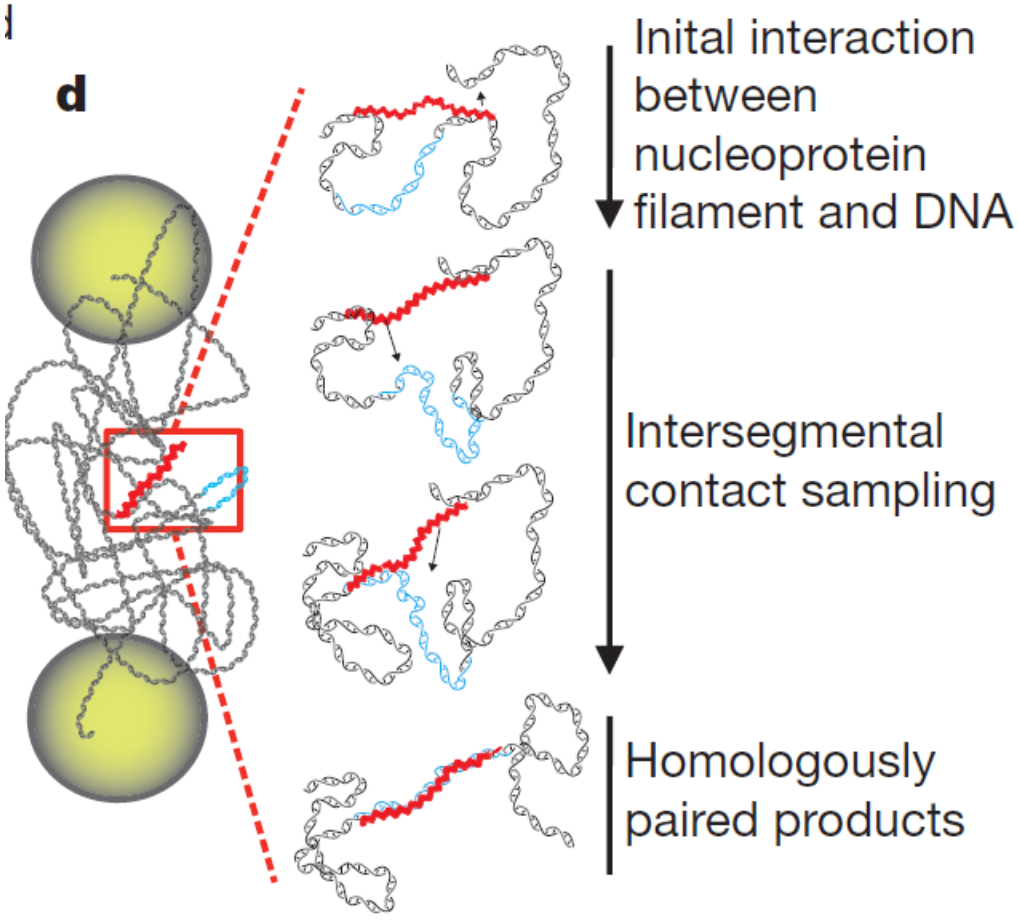

A
0 bp
λ dsDNA molecule
48,502 bp
ssDNA
pairing partners:
162
1,762
430
ssDNA length (nt)
12,449
14,648
24,003
Center of position on λ DNA (bp)
0.26
0.30
0.49
Relative distance on λ DNA


B
Syringe pump
DNA pairing product with
430-nt ssDNA
a Ensemble reaction: DNA pairing product extended on surface
b Doubly attached DNA: DNA pairing in absence or presence of flow
Flow
c Singly attached DNA: DNA pairing in absence of flow; DNA extended by flow after pairing
Flow
d Doubly attached DNA with proximal ends: DNA pairing in absence of flow; DNA extended by flow after pairing


A
Syringe pump
Stage translation
B
Flow
a
Bead channel
b
DNA channel
c
Assembly channel
d, f
De-stain and observation channel
e
Nucleoprotein filament reservoir (no flow)
c
1,762 nt
430 nt
430 nt
430 nt
1,762 nt
Stained with YOYO1 after pairing
Fraction of homologously paired molecules
Center-to-center bead distance (μm)
Fraction of homologously paired molecules
430 nt (2-μm bead distance)
430 nt (6-μm bead distance)
Time (s)
Fraction of homologously paired molecules
1,762 nt (2-μm bead distance)
430 nt (2-μm bead distance)
162 nt (2-μm bead distance)
Time (s)
Fraction of Homologously Paired Molecules
RecA Nucleoprotein Filament Concentration (nM)


a
0
Distance (μm)
16
Time
Incubation with nucleoprotein filaments
Translation into observation channel
Extension of DNA
Two binding events observed
Dissociation of non-homologously paired nucleoprotein filament and release of loop
Stable homologously paired complex persists
Dissociation and loop release


Fraction showing heterologously bound intermediates
0.6
0.4
0.2
0.0
430 nt
1,762 nt
Fraction showing loop-release events
0.6
0.4
0.2
0.0
430 nt
1,762 nt


d
Inital interaction between nucleoprotein filament and DNA
Intersegmental contact sampling
Homologously paired products