

Table 1. RecA Filament Assembly and Synapsis during Replication and Homologous Recombination

Rates and Relevant Physical and Physiological Measurements		Refs
Volume of an <i>E. coli</i> cell	~1 fL (1×10^{-15} l)	[103]
<i>E. coli</i> genome size	4.7 Mb (4.7×10^6 bp)	[104]
Replication rate	650–800 bp/s	[105]
ssDNA generated at replication fork (average Okazaki fragment size)	1000–2000 nucleotides	[106]
Average size of daughter strand gaps	100–800 nucleotides	[107,108]
dsDNA breaks per division (average)	0.1–1	[28,109]
(maximum tolerated)	< 3	[110]
DNA crosslinks per division (maximum tolerated)	50–70	[111]
Oxidative lesions per division	~2000	[28]
Rate of RecBCD resection	1000–2000 bp/s	[14]
Average χ (Chi) frequency	1 per 4500 bp	[14]
Average length of dsDNA resection by RecBCD (<i>in vitro</i>)	30 000 bp	[14]
(<i>in vivo</i>)	10 000 bp	[15]
SSB site size per tetramer	30–70 nucleotides	[37,112]
RecA site size per monomer	3 nucleotides	[29]
Persistence length of dsDNA	~50 nm	[80]
Persistence length of ssDNA	~1 nm	[80,82]
Persistence length of RecA–ssDNA	~900 nm	[44]
Radius of gyration (R_G) for λ dsDNA (48.5 kb)	~900 nm	
RecA nucleation time (rate) ^a , spontaneous	10–60 min (1–6 nuclei/h)	[24]
RecOR-mediated	5–30 min (2–12 nuclei/h)	
RecFOR-mediated	2–10 min (6–30 nuclei/h)	
RecA growth rate ^a , spontaneous	0.3–1.3 RecA monomers/s	[24]
RecOR-mediated	2–6 RecA monomers/s	
RecFOR-mediated	2–6 RecA monomers/s	
RecA K_d for ATP	~15 μ M	[113]
RecA K_d for ATP (+ssDNA)	~2.5 μ M (ssDNA)	
RecA K_m for ATP (+ssDNA)	~20 μ M (ssDNA)	[114]
(+dsDNA)	~100 μ M (dsDNA)	
RecA k_{cat} for ATP (+ssDNA)	~21 per min per RecA	[56]
for dATP (+ssDNA)	~33 per min per RecA	
RecA–ssDNA complex salt titration midpoint ^b , 0.1 mM nucleotide cofactor	255 mM NaCl ~400 mM NaCl (+ATP) 165 mM NaCl (+ADP)	[115]
RecA–dsDNA complex salt titration midpoint ^c , 1 mM nucleotide cofactor	~300 mM NaCl (+ATP) 190 mM NaCl (+ADP)	[116]

^aNucleation and growth rates reported were measured in the presence of ATP γ S (1 μ M RecA, 2 mM ATP γ S, pH 7.5, 37°C, ~8000 nt substrate) [24]. The numbers in Table 1 represent our best estimate for physiologically relevant nucleation and growth rates in the presence of ATP (instead of ATP γ S), which is ten times slower for nucleation and two-thirds slower for growth. However, these rates are affected by temperature, pH, excluded volume, and concentrations of proteins, and monovalent, divalent, and trivalent salts in nonlinear ways. Nonetheless, these estimates are consistent with ensemble experiments with RecFOR [22] and *in vivo* imaging of RecA bundle appearance and growth [13].

^b20 mM Tris acetate, pH 7.5, 4 mM Mg(OAc)₂, 25°C.

^c20 mM MES, pH 6.2, 10 mM MgCl₂, 25°C.

13. Lesterlin, C. *et al.* (2014) RecA bundles mediate homology pairing between distant sisters during DNA break repair. *Nature* 506, 249–253
14. Dillingham, M.S. and Kowalczykowski, S.C. (2008) RecBCD enzyme and the repair of double-stranded DNA breaks. *Microbiol. Mol. Biol. Rev.* 72, 642–671
15. Cockram, C.A. *et al.* (2015) Quantitative genomic analysis of RecA protein binding during DNA double-strand break repair reveals RecBCD action in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 112, E4735–E4742
22. Morimatsu, K. *et al.* (2012) RecFOR proteins target RecA protein to a DNA gap with either DNA or RNA at the 5' terminus: implication for repair of stalled replication forks. *J. Biol. Chem.* 287, 35621–35630
24. Bell, J.C. *et al.* (2012) Direct imaging of RecA nucleation and growth on single molecules of SSB-coated ssDNA. *Nature* 491, 274–278
28. Kuzminov, A. (1999) Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ . *Microbiol. Mol. Biol. Rev.* 63, 751–813
29. Kowalczykowski, S.C. (1991) Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *Annu. Rev. Biophys. Biophys. Chem.* 20, 539–575
37. Raghunathan, S. *et al.* (2000) Structure of the DNA binding domain of *E. coli* SSB bound to ssDNA. *Nat. Struct. Biol.* 7, 648–652
44. Hegner, M. *et al.* (1999) Polymerization and mechanical properties of single RecA–DNA filaments. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10109–10114
56. Menetski, J.P. and Kowalczykowski, S.C. (1989) Enhancement of *Escherichia coli* recA protein enzymatic function by dATP. *Biochemistry* 28, 5871–5881
80. Smith, S.B. *et al.* (1996) Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science* 271, 795–799

82. Murphy, M.C. *et al.* (2004) Probing single-stranded DNA conformational flexibility using fluorescence spectroscopy. *Biophys. J.* 86, 2530–2537
103. Kubitschek, H.E. and Friske, J.A. (1986) Determination of bacterial cell volume with the Coulter Counter. *J. Bacteriol.* 168, 1466–1467
104. Blattner, F.R. *et al.* (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* 277, 1453–1462
105. Reyes-Lamothe, R. *et al.* (2010) Stoichiometry and architecture of active DNA replication machinery in *Escherichia coli*. *Science* 328, 498–501
106. Kornberg, A. and Baker, T.A. (1992) *DNA Replication*, W.H. Freeman and Co
107. Iyer, V.N. and Rupp, W.D. (1971) Usefulness of benzoylated naphthoylated DEAE-cellulose to distinguish and fractionate double-stranded DNA bearing different extents of single-stranded regions. *Biochim. Biophys. Acta* 228, 117–126
108. Wang, T.C. and Chen, S.H. (1992) Similar-sized daughter-strand gaps are produced in the leading and lagging strands of DNA in UV-irradiated *E. coli* *uvrA* cells. *Biochem. Biophys. Res. Commun.* 184, 1496–1503
109. Pennington, J.M. and Rosenberg, S.M. (2007) Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nat. Genet.* 39, 797–802
110. Krasin, F. and Hutchinson, F. (1977) Repair of DNA double-strand breaks in *Escherichia coli*, which requires *recA* function and the presence of a duplicate genome. *J. Mol. Biol.* 116, 81–98
111. Sinden, R.R. and Cole, R.S. (1978) Repair of cross-linked DNA and survival of *Escherichia coli* treated with psoralen and light: effects of mutations influencing genetic recombination and DNA metabolism. *J. Bacteriol.* 136, 538–547

112. Lohman, T.M. and Ferrari, M.E. (1994) *Escherichia coli* single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities. *Annu. Rev. Biochem.* 63, 527–570
113. Kowalczykowski, S.C. (1986) Interaction of recA protein with a photoaffinity analogue of ATP, 8-azido-ATP: determination of nucleotide cofactor binding parameters and of the relationship between ATP binding and ATP hydrolysis. *Biochemistry* 25, 5872–5881
114. Weinstock, G.M. *et al.* (1981) Hydrolysis of nucleoside triphosphates catalyzed by the recA protein of *Escherichia coli*. Steady state kinetic analysis of ATP hydrolysis. *J. Biol. Chem.* 256, 8845–8849
115. Menetski, J.P. and Kowalczykowski, S.C. (1985) Interaction of recA protein with single-stranded DNA. Quantitative aspects of binding affinity modulation by nucleotide cofactors. *J. Mol. Biol.* 181, 281–295
116. Zaitsev, E.N. and Kowalczykowski, S.C. (1998) Binding of double-stranded DNA by *Escherichia coli* RecA protein monitored by a fluorescent dye displacement assay. *Nucleic Acids Res.* 26, 650–654