*Bacillus subtilis* RecA with DprA-SsbA antagonizes RecX function during natural transformation

**Shimin Le[1,2,†], Ester Serrano[3,†], Ryo Kawamura[1,2], Begoña Carrasco[3], Jie Yan[1,2,*] and Juan C. Alonso[3,*]**

[1]Department of Physics and [2]Mechanobiology Institute, National University of Singapore, Singapore 117411; [3]Department of Microbial Biotechnology, Centro Nacional de Biotecnología (CNB-CSIC), Darwin 3, Cantoblanco, 28049 Madrid, Spain

Table S1. Role of RecX in chromosomal and plasmid transformation

| Strain[a] | Relevant genotype | Normalized plasmid transformation | Normalized chromosomal transformation |
|---|---|---|---|
| BG214 | *rec*$^+$ | 100 (2.4 x $10^4$) | 100 (4.0 x $10^6$) |
| BG190 | Δ*recA* | 98 | <0.01 |
| BG1163 | Δ*dprA* | 2.3 | 1.4 |
| BG1065 | Δ*recX* | 1.7 | 0.5 |
| BG1291 | Δ*recA* Δ*dprA* | 1.0 | <0.01 |
| BG1147 | Δ*recA* Δ*recX* | 57 | <0.01 |
| BG1609 | Δ*recX* Δ*dprA* | <1.6 | <0.1 |

[a]All strains are isogenic with BG214 (*trpCE metA*5 *amyE1 ytsJ*1 *rsbV*37 *xre*1 *xkd*A1 *att*$^{SP\beta}$ *att*$^{ICEBs1}$). Competent *B. subtilis* cells auxotrophic for methionine were transformed with chromosomal DNA from prototroph SB19 strain. The yield of *met*$^+$ (chromosomal transformation) and neomycin-resistant transformants (plasmid pUB110 transformation) was corrected for DNA uptake and cell viability, and the values obtained were normalized to that of the *rec*$^+$ strain, taken as 100 (in parentheses, the number of transformants obtained per 0.1 μg DNA ml$^{-1}$). Results are the mean (± 10% SEM) of at least seven independent experiments.

**Figure legends**

Fig. S1. Effect of RecA on RecA·dATP nucleation and polymerization. *A*, ssDNA was pre-incubated with RecX, SsbA, SsbB or RecX and a SSB protein (5 min), followed by RecA·dATP, and dATPase activity was measured for 30 min. *B*, Circular ssDNA was pre-incubated with RecA or RecA and a SSB protein (5 min), followed by RecA, and dATPase activity measured for 30 min. The amount of dATP hydrolyzed was calculated. All reactions were repeated at least three times with similar results.

Fig. S2. RecX leads to unstable nucleation and polymerization of RecA filament in the presence of ATP. *A*, extension time traces of ssDNA after introduction of the RecX RecA mixture in buffer C containing ATP at ~4 pN. *B*, extension time traces of ssDNA after introduction of the RecX and RecA mixture at ~9 pN. *C*-*D*, ATPγS (*C*) or dATP (*D*) facilitates RecA filament stability in the presence of RecX. *C*, typical extension time traces of preformed RecA filament in the presence of RecA and RecX with ATPγS at ~3 pN. *D*, typical extension time traces of preformed RecA filament in the presence of RecA RecX and SsbA with dATP at ~4 pN. Red lines in A-D are the 40-point smooth of the raw data.

Fig. S3. RecX leads to de-polymerization of RecA·ATP filament at low forces, while high forces facilitate re-polymerization of RecA·ATP filament. *A*-*B*, typical extension time traces of net depolymerization of RecA filaments in the presence of two RecX concentrations and RecA at ~3 pN. Red lines indicate the 40-point smooth of raw data (colored). *C*, typical extension time traces of partially depolymerized RecA after increasing force to ~12 pN in the presence of RecX. *D*, typical extension time traces of partially depolymerized RecA filaments cycling between ~3 pN (red) and >25 pN (blue) in the presence of RecA, RecX and SsbA.

Figure S1

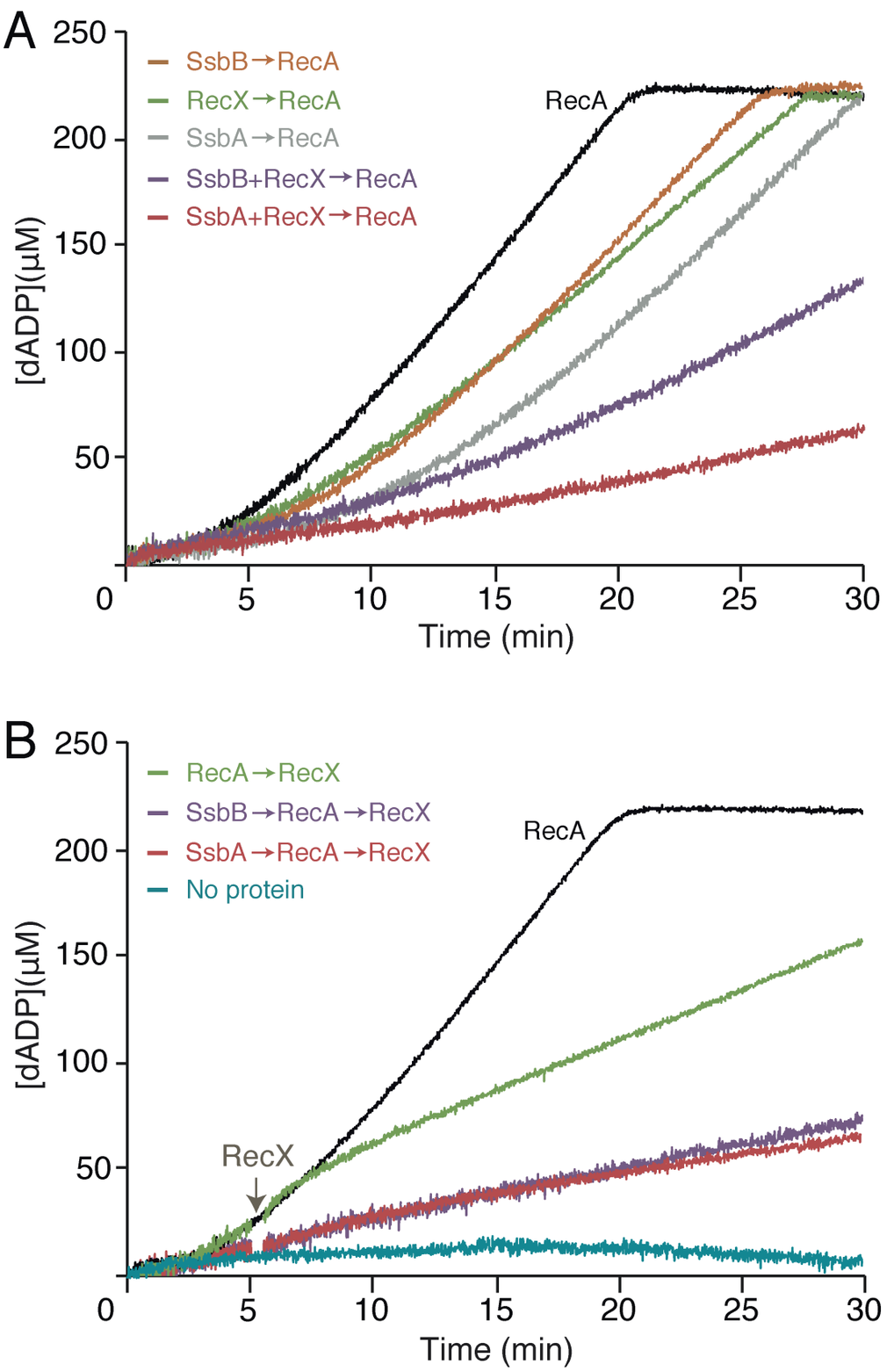

A
250
200
150
100
50
0
[dADP](μM)
SsbB→RecA
RecX→RecA
SsbA→RecA
SsbB+RecX→RecA
SsbA+RecX→RecA
RecA
5
10
15
20
25
30
Time (min)
B
250
200
150
100
50
0
[dADP](μM)
RecA→RecX
SsbB→RecA→RecX
SsbA→RecA→RecX
No protein
RecA
RecX
5
10
15
20
25
30
Time (min)

Figure S2

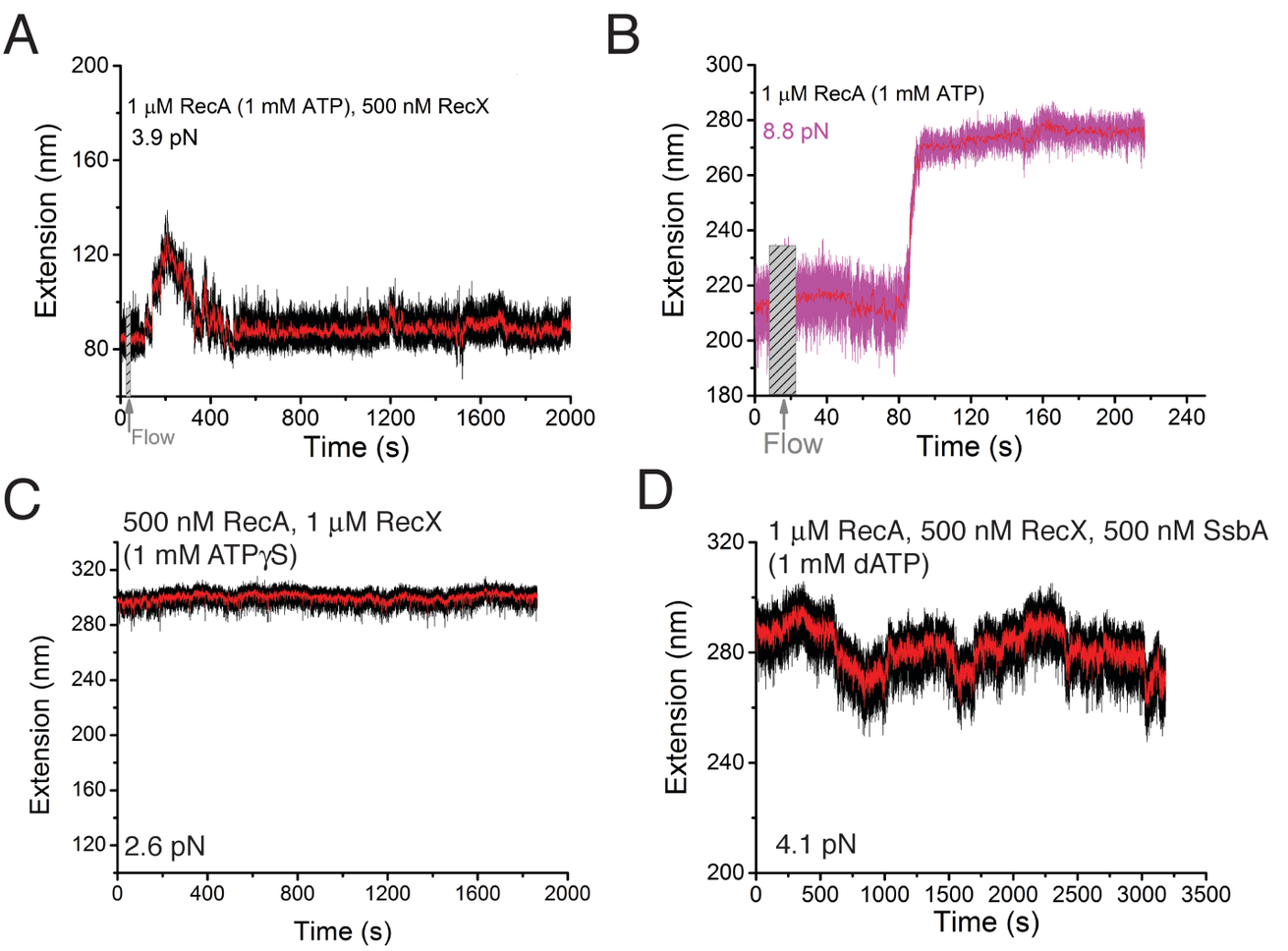
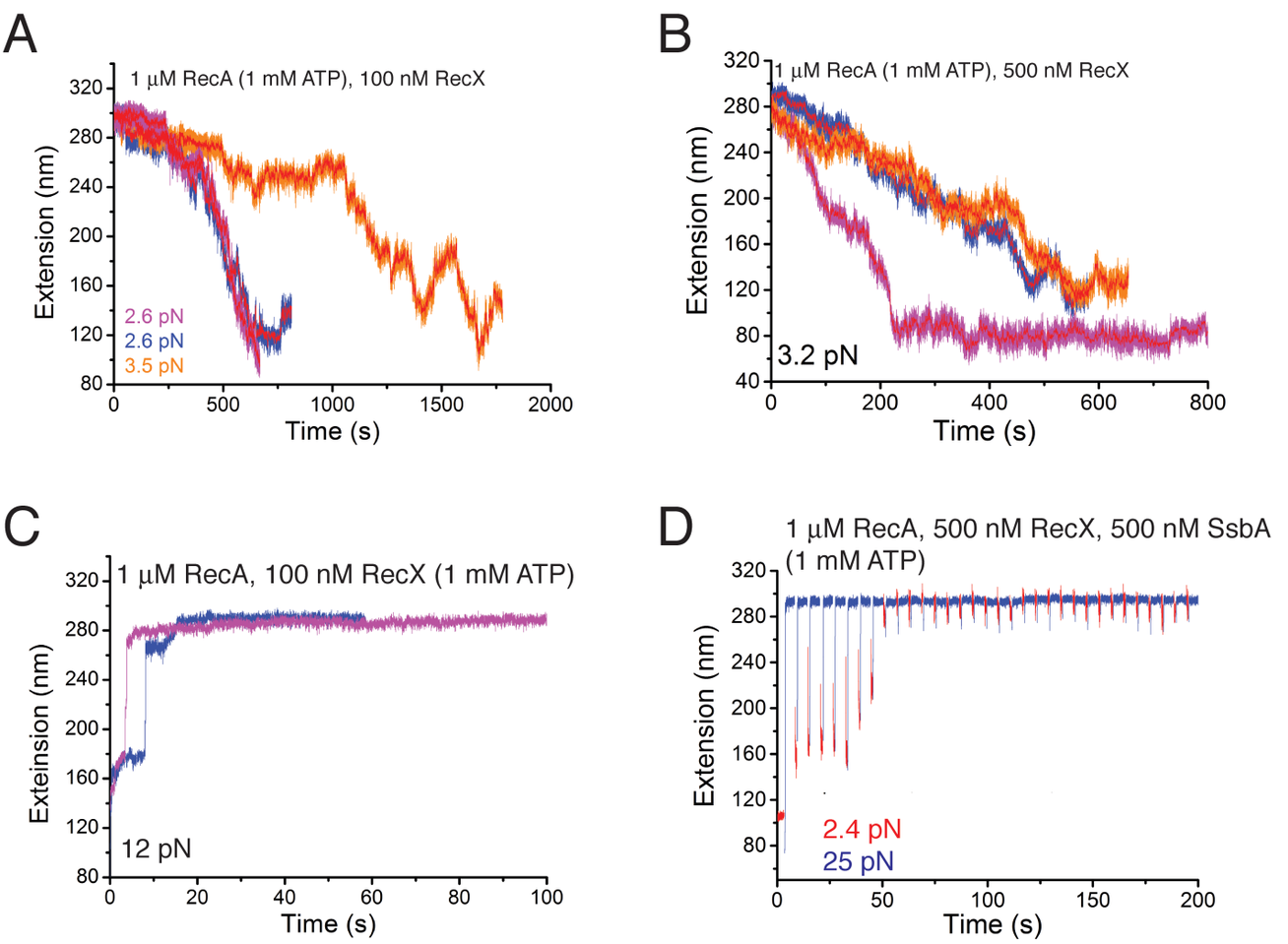

A
1 μM RecA (1 mM ATP), 500 nM RecX
3.9 pN
Extension (nm)
Time (s)
Flow
B
1 μM RecA (1 mM ATP)
8.8 pN
Extension (nm)
Time (s)
Flow
C
500 nM RecA, 1 μM RecX
(1 mM ATPγS)
2.6 pN
Extension (nm)
Time (s)
D
1 μM RecA, 500 nM RecX, 500 nM SsbA
(1 mM dATP)
4.1 pN
Extension (nm)
Time (s)

Figure S3


A
1 μM RecA (1 mM ATP), 100 nM RecX
Extension (nm)
Time (s)
2.6 pN
2.6 pN
3.5 pN
B
1 μM RecA (1 mM ATP), 500 nM RecX
Extension (nm)
Time (s)
3.2 pN
C
1 μM RecA, 100 nM RecX (1 mM ATP)
Exteinsion (nm)
Time (s)
12 pN
D
1 μM RecA, 500 nM RecX, 500 nM SsbA
(1 mM ATP)
Extension (nm)
Time (s)
2.4 pN
25 pN