Science Advances
AAAS

Supplementary Materials for

Intein splicing efficiency and RadA levels can control the mode of archaeal DNA replication

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Sci. Adv. **10**, eadp4995 (2024)
DOI: 10.1126/sciadv.adp4995

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Figs. S1 to S4

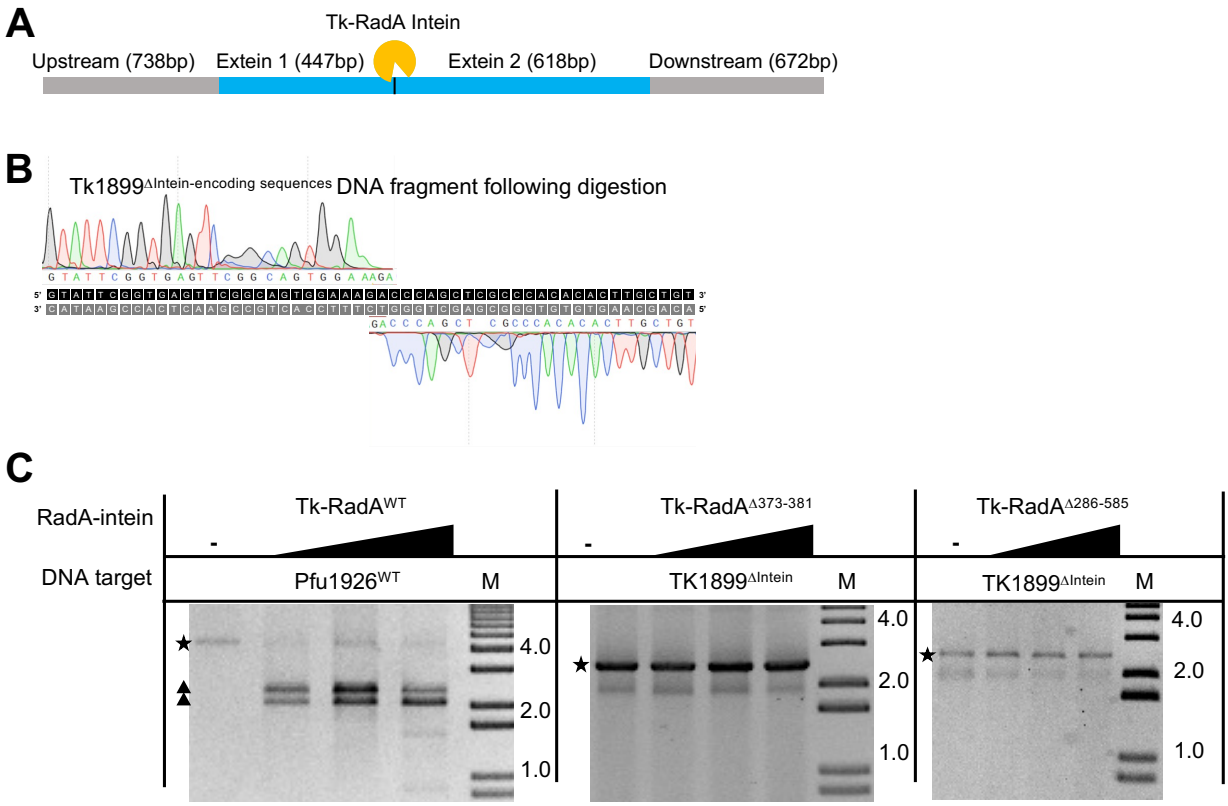
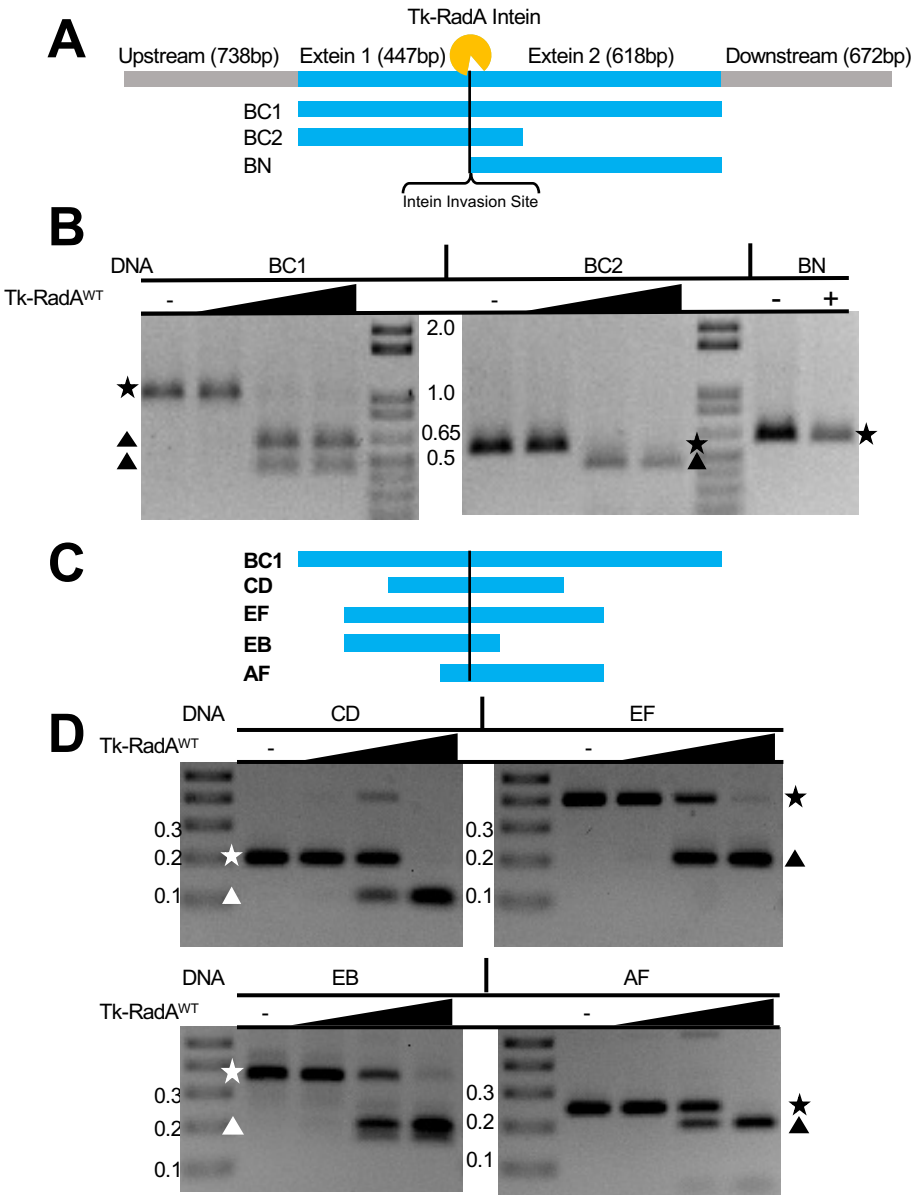


Fig. S1. Tk-RadA^{WT} contains a self-splicing intein with active homing endonuclease activities capable of cleaving intein-less alleles.

(A) Diagrams of the DNA fragments generated and tested to confirm the cleavage site of the Tk-RadA HEN activity. Grey and blue bars denote sequences surrounding and encoding an intein-less allele of TK1899, respectively. The black line denotes the presumptinve HEN cut site between the extein-encoding sequences. (**B)** Cleavage fragments of the amplicon containing the Tk1899$^{\Delta Intein\text{-}encoding\ sequences}$ DNA sequence were Sanger sequenced to identify the exact position of cleavage on each strand. **(C)** *In vitro* HEN assays demonstrate that Tk-RadA^{WT} can efficiently cleave amplicons of the native, intein-less allele of RadA from the closely related species *Pyrococcus furiosus* (Pfu1926^{WT}; left). HEN activities are compromised in Tk-RadA variants wherein active site residues are missing or lack its HEN domain, RadA$^{\Delta 373\text{-}381}$ (middle) and RadA$^{\Delta 286\text{-}585}$ (right) respectively. Intact DNA target and cleaved DNA target(s) are denoted as a star and triangles, respectively.


A
Tk-RadA Intein
Upstream (738bp)
Extein 1 (447bp)
Extein 2 (618bp)
Downstream (672bp)
BC1
BC2
BN
Intein Invasion Site
B
DNA
BC1
BC2
BN
Tk-RadA^WT
-
-
-
+
2.0
1.0
0.65
0.5
C
BC1
CD
EF
EB
AF
D
DNA
CD
EF
Tk-RadA^WT
-
-
0.3
0.2
0.1
DNA
EB
AF
Tk-RadA^WT
-
-
0.3
0.2
0.1

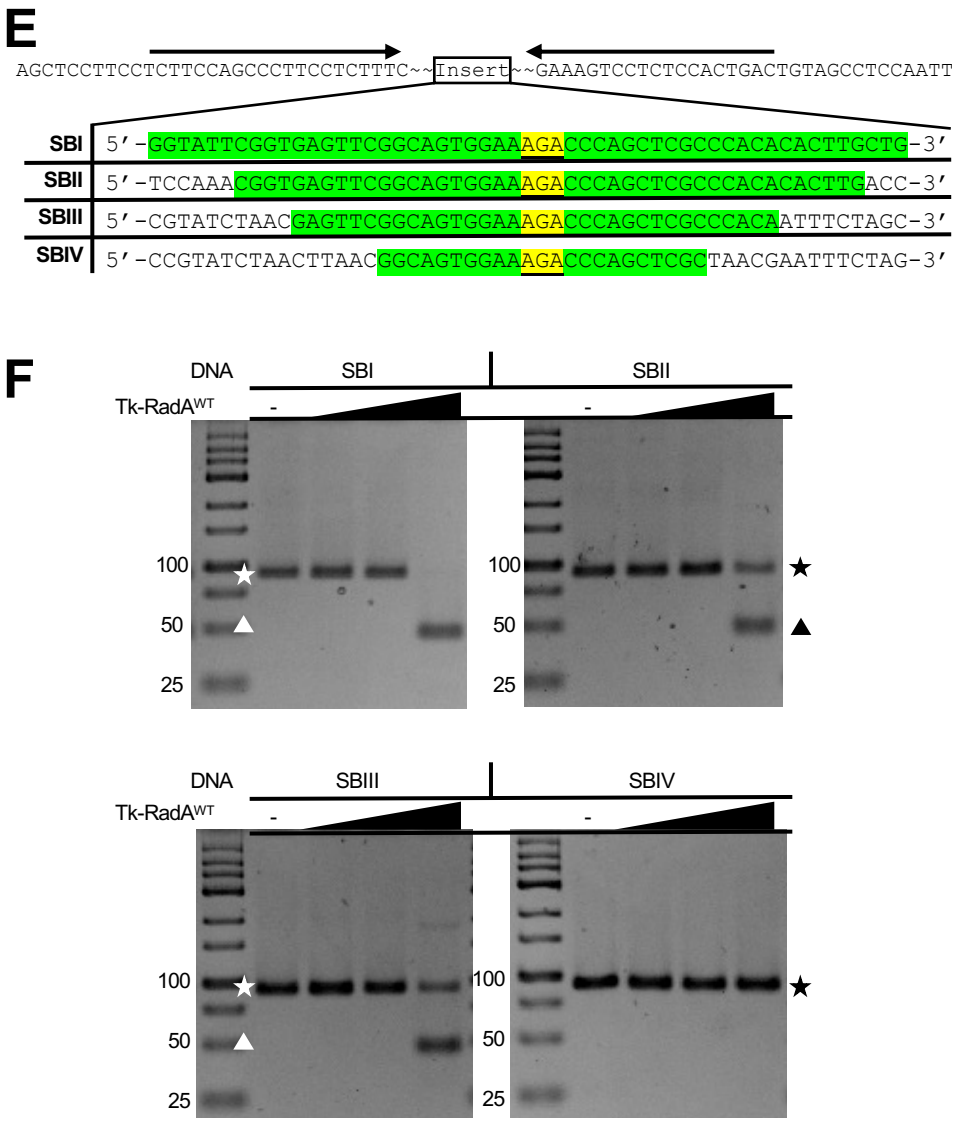


Fig. S2. Defining the minimum recognition sequence necessary for HEN-initiated DNA cleavage of intein-less alleles.

(A, C, E) Diagrams and sequences of the DNA fragments generated and tested to narrow the recognition site of Tk-RadA HEN activity. Grey and blue bars denote surrounding and TK1899 intein-encoding sequences, respectively. The black line denotes the HEN cut site. Artificial substrates with decreasing sequences derived from TK1899 (highlighted in green) were inserted into the Blue Heron pUC MinusMCS vector, from which amplicons were generated to assay HEN activities. (**B, D, F)** HEN assays monitor the ability of purified Tk-RadA^{WT} protein to recognize amplicons containing targeting sequences and cleave the DNA fragments. HEN activities of Tk-RadA^{WT} are detected against DNA sequences containing at least 30 bp of the cut site but not detected when only 20 bp sequence surrounding the cut site are available within the amplicon. Intact DNA target and cleaved DNA target(s) are denoted as a star and triangles, respectively.

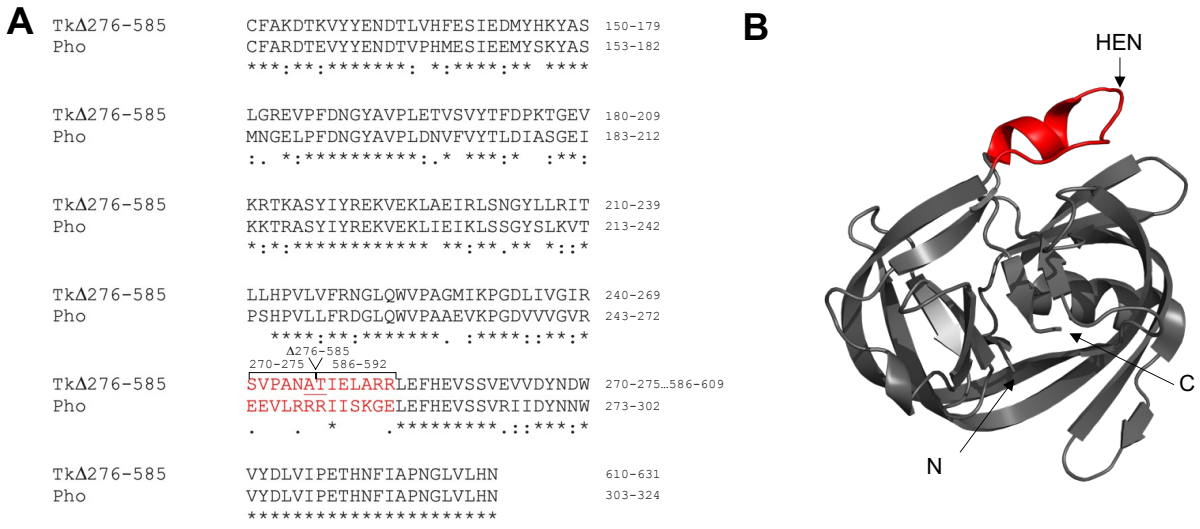


Fig. S3. Loop residues in the *P. horikoshii* RadA intein are important for splicing.
(A, B) Sequence alignment of the Tk-RadA$^{\Delta 276\text{-}585}$ and the Ph-RadA inteins reveals substantial congruence except for a 14 aa (highlighted in red) patch where the HEN domain of Tk-RadA is normally inserted. The divergent residues are known to form a short helix and loop within the atomic structure of the *P. horikoshii* RadA-intein (right, in cartoon) that impacts the temperature-dependence of intein splicing.

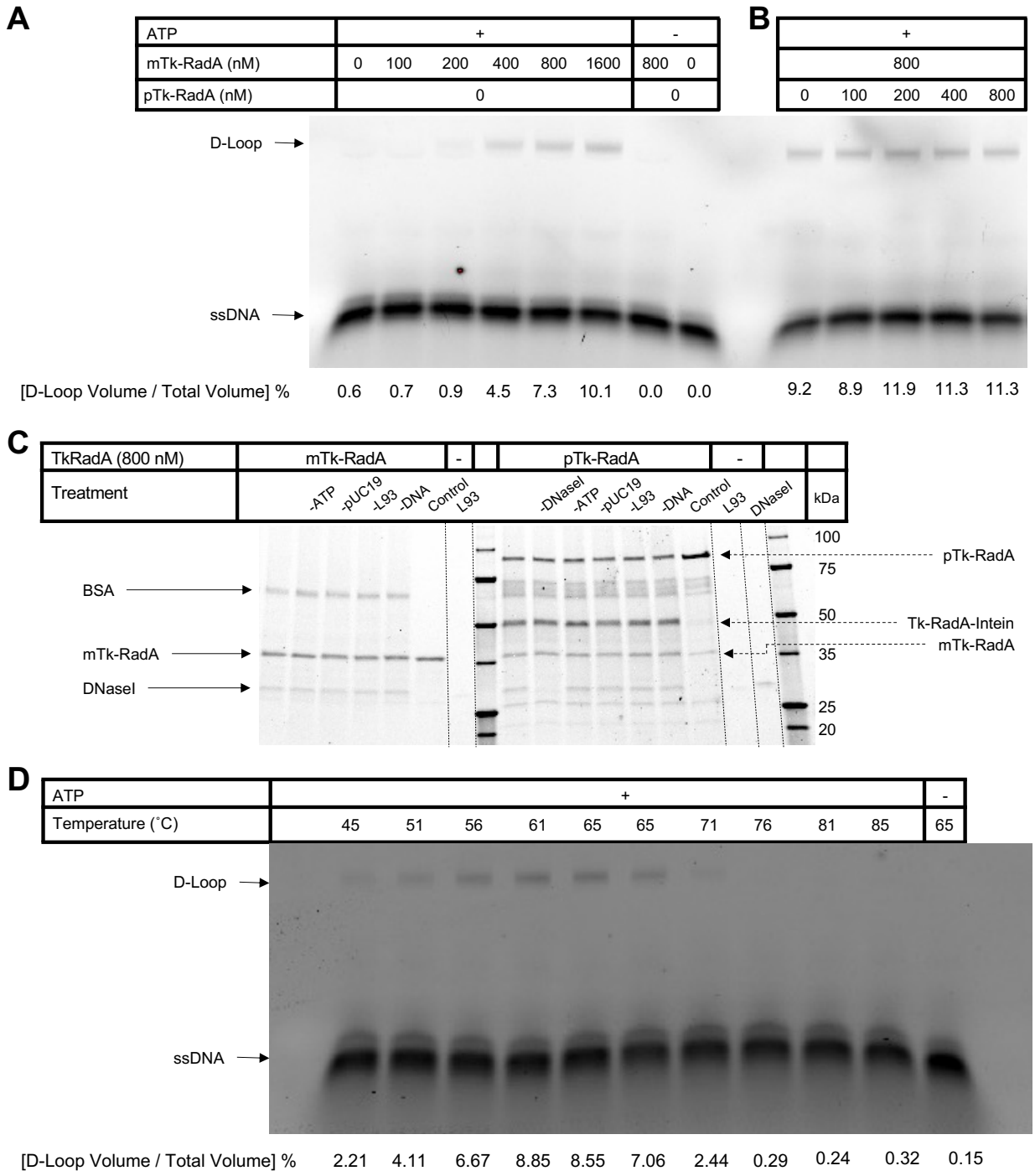


Fig. S4. Precursor Tk-RadA does not inhibit recombinase activity of mature Tk-RadA. Native TBE agarose gel electrophoresis visualized by fluorescence demonstrating mTk-RadA-mediated D-loop formation. **A-B.** mTk-RadA functions as a recombinase, catalyzing a protein-concentration and ATP-dependent invasion of a ssDNA into the supercoiled pUC19 plasmid forming a D-loop (**A**). Addition of pTk-RadA does not impact the efficiency of mTk-RadA recombinase activities (**B**). D-loop formation was quantified as the percentage of fluorescent oligo with retarded migration in each lane. **C.** SDS-PAGE of the purified proteins used in the D-

loop formation assays reveals an elevated splicing efficiency for pTk-RadA under our D-loop assay conditions. **D.** D-loop formation assay incubated at various temperatures, 45 – 85 ˚C, with a constant mTk-RadA concentration kept at 800 nM. D-loop formation were quantified from the ratio of [D-Loop Volume/Total Volume]%.