Supporting Information

Mechanism of single-stranded DNA activation of recombinase intein splicing

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Experimental Procedures

Plasmid construction and mutagenesis: pET47b-RadA (Δ1-115 codons) was subcloned from pET45b-RadA (Δ1-115 codons) (14) using BamHI/XhoI restriction sites, resulting in an in-frame N-terminal His-tag. *Pho* RadA N172A/T+1A (pET47b), C1A (pET45b) and C+1 (pET47b) were constructed using the Q5 site-directed mutagenesis kit (New England Biolabs). Primers IDT5967 (5'-TGTTCTCCATGCGGCGCAGCTAGCCC-3') and IDT5968 (5'-AGTCCATTGGGAGCTATG-3') were used to make N172A/T+1A, IDT5965 (5'-TAGTGGGAAGGCCTTTGCTAGGGATAC-3') and IDT5966 (5'-CCGAACTCCCCAAAGACT-3') were used to make C1A, and IDT5961 (5'-TCTCCATAATTGCCAGCTAGCCCATAC-3') and IDT5962 (5'-ACAAGTCCATTGGGAGCT-3') were used to make C+1.

Protein expression and purification: All proteins were expressed in *E. coli BL21*(DE3) (Novagen). Cells were grown at 37ºC to an optical density of ~0.5 at 600 nm, the

temperature was reduced to 16ºC, expression was induced by addition of 0.5 mM isopropyl ß-D-1-thiogalactopyranoside, and the cells were grown an additional ~16 hours. Proteins were purified by immobilized metal affinity chromatography using Ni-NTA resin (Qiagen) as described previously (14). Due to remaining impurities following Ni-NTA affinity chromatography for RadA N172A/T+1A and C1A, these proteins were further purified by cleavage of the His-tag followed by a second round of negative Ni-NTA chromatography. HRV 3C protease (Pierce) was used for N172A/T+1A and Enterokinase (Abcam) was used for C1A.

Splicing, cleavage and disulfide bonding assays: Splicing assays using WT *Pho* RadA were performed as previously described (14), except that the final concentration of protein was 0.25 mg/mL. NTC, CTC, and disulfide bonding assays using *Pho* RadA mutants were also performed under these conditions, except that 20 mM DTT was present in NTC assays with RadA N172A/T+1A and 20 mM TCEP was used in disulfide bonding assays with RadA C+1, where indicated. Samples were separated by SDS-PAGE, which was non-reducing unless otherwise noted. Times and presence or absence of ssDNA (M13mp18; New England Biolabs) are indicated in the figures and legends. All assays were performed at 63ºC unless otherwise noted. Splicing of WT RadA was calculated as $[(LE/LE+P)_{Tx} - (LE/LE+P)_{T0}]/[1 - (LE/LE+P)_{T0}]$. N-terminal cleavage was calculated as the level of $I\text{-}C_{Tx} - I\text{-}C_{T0,}$ normalized to 1 where 1 is the maximum I-C detected. C-terminal cleavage was calculated as the level of $C_{Tx} - C_{T0,}$ normalized to 1 where 1 is the maximum C detected. Disulfide bond formation was calculated as $P_{oxidized}/(P_{oxidized} + P_{reduced})$.