Supporting Text

DdcA antagonizes a bacterial DNA damage checkpoint

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Running Title: DdcA inhibits a DNA damage checkpoint

Supporting Results

The PDZ domains of DdcP and CtpA have different functions *in vivo*

The influence of a PDZ domain on protease activity has been found to be both positive and negative (Clausen, Kaiser, Huber, & Ehrmann, 2011). Both DdcP and CtpA have PDZ domains, but the function of the PDZ domains in YneA degradation is unknown. We performed a PSI-BLAST search using the PDZ domain of DdcP against the *E. coli* protein database. We found that the PDZ domain of DdcP was most similar to the PDZ domain of DegS and PDZ1 from DegP (Fig S6A), both of which have been reported to inhibit protease activity (Krojer, Sawa, Huber, & Clausen, 2010; Sohn, Grant, & Sauer, 2007; Spiess, Beil, & Ehrmann, 1999; Walsh, Alba, Bose, Gross, & Sauer, 2003). We asked if CtpA would yield the same PSI-BLAST result, so we repeated our search using the PDZ domain of CtpA. CtpA was most similar to the PDZ domain of the C-terminal processing protease Prc (also known as Tsp; Fig S6B), which was expected because CtpA and its homolog CtpB return Prc in a standard BLAST search (data not shown). Intriguingly, the PDZ domains of CtpB and Prc have been reported to function in substrate recognition (Beebe et al., 2000; Mastny et al., 2013). Together, our homology searches predict that the PDZ domains of DdcP and CtpA may have different functions.

To test our hypothesis that DdcP and CtpA have PDZ domains with different functions, we constructed ΔPDZ constructs for both DdcP and CtpA (Fig S6C) at the native locus of each gene. We tested for MMC sensitivity using a spot titer assay. Deletion of the PDZ domain of DdcP had no effect on MMC sensitivity (Fig S6D), suggesting that this PDZ domain is inhibitory similar to the PDZ domain of DegS. Deletion of the PDZ domain of CtpA resulted in a mutant that was sensitive to MMC, but not to the same extent as deletion of *ctpA* (Fig S6D), suggesting that the PDZ domain of CtpA functions in substrate recognition similar to Prc and CtpB. As a control, we verified that the ΔPDZ variants were stably expressed *in vivo* (Fig S6E). Taken together, our results support the bioinformatics predictions, and we conclude that the PDZ domains of DdcP and CtpA have different functions in regulating protease activity.

Supporting Tables

Supporting Table 1. Plasmids used in this study

Plasmid number	Plasmid name	Reference/Source
pKT25	pKT25	Euromedex (EUP-25C)
pUT18C	pUT18C	Euromedex (EUP-18C)
pDR244	pDR244	*Bacillus* Genetic Stock Center
pPB41	pPB41	(Burby & Simmons, 2017a, 2017b)
pPB47	pPB47	(Burby, Simmons, Schroeder, & Simmons, 2018)
pPB73	pPB41-CRISPR::*uvrB*	(Burby et al., 2018)
pPB84	pPB73-Δ*uvrAB* editing template	This Study
pPB108	pPB47-*amyE::*P_{xyl}*-ddcP-cam*^{R}	(Burby et al., 2018)
pPB122	pPB115-Δ*ddcA* editing template	(Burby et al., 2018)
pPB147	pPB47-*amyE::*P_{xyl}*-ddcA-cam*^{R}	This study
pPB184	pPB47-*amyE::*P_{xyl}*-ctpA-cam*^{R}	(Burby et al., 2018)
pPB192	pPB47-*amyE::*P_{xyl}*-yneA-cam*^{R}	This study
pPB216	pPB47--*amyE::*P_{xyl}*-ddcPΔTM-cam*^{R}	This study
pPB235	pPB41-CRISPR::*ddcP-PDZ*	This study
pPB236	pPB41-CRISPR::*ctpA-PDZ*	This study
pPB245	pPB235-*ddcP*Δ*PDZ* editing template	This study
pPB246	pPB236-*ctpA*Δ*PDZ* editing template	This study
pPB254	pPB47-*amyE::*P_{xyl}*-gfp-ddcA-cam*^{R}	This study
pPB255	pPB47-*amyE::*P_{xyl}*-ddcA-gfp-cam*^{R}	This study
pPB257	pPB47-*amyE::*P_{xyl}*-gfp-yneA-cam*^{R}	This study
pPB267	pUT18-YneA	(Burby et al., 2018)
pPB268	pUT18-YneAΔN	(Burby et al., 2018)
pPB269	PKT25-DdcA	This study
pPB270	pKT25-DdcP-S234A	(Burby et al., 2018)
pPB271	pKT25-CtpA-S297A	(Burby et al., 2018)

Supporting Table 2. Oligonucleotides used in this study

Primer name	Sequence
oPEB116	Ctctcgtttcatcggtatcattac
oPEB117	Cgcttcgttaatacagatgtaggt
oPEB217	GAACCTCATTACGAATTCAGCATGC
oPEB218	GAATGGCGATTTTCGTTCGTGAATAC
oPEB227	CCGTCAATTGTCTGATTCGTTA
oPEB232	GCTGTAGGCATAGGCTTGGTTATG
oPEB234	GTATTCACGAACGAAAATCGCCATTCCTAGCAGCACGCCATAGTGACTG
oPEB253	GAAGGGTAGTCCAGAAGATAACGA
oPEB345	Actcctttgtttatccaccgaac
oPEB348	TTATTTTTGACACCAGACCAACTG
oPEB370	cacctacatctgtattaacgaagcgTCAATGGGGAAGAGAACCGCTTAAG
oPEB377	ggtaatgataccgatgaaacgagagAACAAAATTCTCCAGTCTTCACATCG
oPEB383	Atgtatacctccttaggatcccatttcc
oPEB422	GCATAACCAAGCCTATGCCTACAGCgaagactttgtaattgcggaaaac
oPEB424	Agaatgaatcgtgaaatgatcacc
oPEB428	GCATGCTGAATTCGTAATGAGGTTCtagtctcttgaagctggttgtcct
oPEB432	Acggatcgatatgattctctaagc
oPEB434	Ctgaggagggttttgttgattac
oPEB435	Taacagagggttaaaaataagcctccgtttctttaacg
oPEB436	Ttcgttaaagaaacggaggcttatttttaaccctctgttaagaggggacagcttgtcggcaa gtccatccttgggcttagcaggcaagcttttttctttac
oPEB477	GCATGCTGAATTCGTAATGAGGTTCagcagtacctgtcctcttgattct
oPEB480	GCATAACCAAGCCTATGCCTACAGCccagtatgtgacctcgattctaac
oPEB481	TACATAAGCACCAAATTGAAGTGG
oPEB482	Agaaacagcacagcttattgatga
oPEB483	GAGAAAAGATTGTGTTCCGAAAAG
oPEB492	Tgatgttctttttcctcctattcg
oPEB493	Acgatattgccgtattcctcttat
oPEB557	taaCGGTTTCCATATGGGGATTGGTG
oPEB561	ACCAATCCCCATATGGAAACCGttaTCAGGTGCTTTTCGCTTTCAGCTT
oPEB588	GCATGCTGAATTCGTAATGAGGTTCcccctcctatcctgactttctatc
oPEB591	GCATAACCAAGCCTATGCCTACAGCtatgggtcattatgctgtttatgg
oPEB592	Acgactttaccttgatggtttttg
oPEB593	Gttgctctttacacattcttcagc
oPEB594	Ggatacagcaaatgtcctaaataagc
oPEB601	Cgtgcttatgaatatatgggattg
oPEB602	Aacaagctcttcacgcaatttag
oPEB706	aaatgggatcctaaggaggtatacatATGACGCATGACAAGAAAAACGCA
oPEB707	CACCAATCCCCATATGGAAACCGttaTTACAAATAAGAAATTTCTTCAATATCTTTTAAC

oPEB708	AGAAGTCAAAAGCACCATTGAAG
oPEB818	ACCAATCCCCATATGGAAACCGttaTTACATTTCTTTTTTCAGTGTTTCAATTGC
oPEB854	aatgggatcctaaggaggtatacatATGAGTAAAGAATCTATTATTTTTGTCGGT
oPEB855	CACCAATCCCCATATGGAAACCGttaCTATCTTACAGTTGCTAATTCATATG
oPEB887	aatgggatcctaaggaggtatacatTTGACAGAGCTTGCTTCATTGATAA
oPEB911	gaaatgggatcctaaggaggtatacatTTGAGCACAGGAGACAGCAAGTTCGAC
oPEB984	aaacAGAAGTCGGTGATAAAATCATCAGCGCAGAg
oPEB985	aaaacTCTGCGCTGATGATTTTATCACCGACTTCT
oPEB986	TTCCTGCCCGATCCGGCTCATCAGGGCTGACTTTTTTTCCTGCCTTTTGA
oPEB987	ATCAAAAGGCAGGAAAAAAAGTCAGCCCTGATGAGCCGGATCGGGCAG
oPEB988	aaacGAATGTAAATGAAGCCGTCGCTTTAATCCGg
oPEB989	aaaacCGGATTAAAGCGACGGCTTCATTTACATTC
oPEB990	CATCTCTGAATAGACAGTCTCTACAGGTGCTGAAATCGTCTCGTCGAATG
oPEB991	AATCATTCGACGAGACGATTTCAGCACCTGTAGAGACTGTCTATTCAGAG
oPEB998	aaatgggatcctaaggaggtatacatATGAGTAAAGGAGAAGAACTTTTCAC
oPEB999	TTTTGCGTTTTTCTTGTCATGCGTCATGAGCTCAGAGCGGTAAGCGTAA
oPEB1000	GTGGTTACGCTTACCGCTCTGAGCTCATGACGCATGACAAGAAAAACGCA
oPEB1001	AAAGATATTGAAGAAATTTCTTATTTGGGTGAAGGTCAAGGACAAGGCCA
oPEB1002	CCACCAATCCCCATATGGAAACCGttaTTATTTGTATAGTTCATCCATGCCATGTG
oPEB1003	TCCTTGGCCTTGTCCTTGACCTTCACCCAAATAAGAAATTTCTTCAATATCTTTTAAC
oPEB1005	GAGCTCAGAGCGGTAAGCGTAAC
oPEB1006	GGACGTGGTTACGCTTACCGCTCTGAGCTCATGAGTAAAGAATCTATTATTTTTGTCGGT
oPEB1014	TTGGCCTTGTCCTTGACCTTCACCGGGATCCTCTAGAGTCGACCCTG
oPEB1015	TAActaagaattcggccgtcgttt
oPEB1021	ATTATGCCGCATCTGTCCAACT
oPEB1022	Gcaaggcgattaagttgggtaa
oPEB1037	AGGATCCCGGTGAAGGTCAAGGACAAGGCCAAATGACGCATGACAAGAAAAACGCA
oPEB1038	taaaacgacggccgaattcttagTTATTACAAATAAGAAATTTCTTCAATATCTTTTAAC

Supporting Experimental Procedures

Bacterial two-hybrid assays

Bacterial two-hybrid assays were performed as previously described (Burby et al., 2018; Karimova, Dautin, & Ladant, 2005; Karimova, Pidoux, Ullmann, & Ladant, 1998).

Strain construction

Individual strains were generated using CRISPR/Cas9 genome editing or double cross-over recombination as previously described (Burby et al., 2018). Strains using P_{xyl} at the *amyE* locus have a chloramphenicol resistance cassette and were selected for using LB agar + 5 μg/mL chloramphenicol.

PEB309 (Δ*uvrAB*): PY79 was transformed with editing plasmid pPB84 to delete *uvrAB*. Deletion of *uvrAB* was verified via PCR genotyping using oPEB424/432.

PEB495 (Δ*ddcA,* Δ*yneA::erm*): PEB357 (Δ*ddcA*) was transformed with chromosomal DNA purified from PEB432 (Δ*yneA::erm*, BKE17860 from *Bacillus* Genetic Stock Center). Replacement of *yneA* with the *erm* cassette was verified via PCR genotyping using oPEB492/493.

PEB497 (Δ*uvrAB,* Δ*ddcA*): PEB309 was transformed with editing plasmid pPB122 to delete *ddcA*. Deletion of *ddcA* was verified via PCR genotyping using oPEB601/602.

PEB499 (Δ*ddcP,* Δ*ddcA*): PEB324 was transformed with editing plasmid pPB122 to delete *ddcA*. Deletion of *ddcA* was verified via PCR genotyping using oPEB601/602.

PEB503 (Δ*ddcA*, *amyE::*P_{xyl}*-ddcA*): PEB357 was transformed with pPB147 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB579 (Δ*ctpA,* Δ*ddcA*): PEB355 was transformed with editing plasmid pPB122 to delete *ddcA*. Deletion of *ddcA* was verified via PCR genotyping using oPEB601/602.

PEB587 (Δ*ddcA,* Δ*yneA::loxP*): The *erm* cassette at the *yneA* locus was removed via Cre recombinase by transforming PEB495 with pDR244, which was subsequently evicted. The absence of the *erm* cassette was verified by testing for sensitivity to erythromycin and by PCR genotyping using oPEB492/493.

PEB639 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*): PEB555 was transformed with editing plasmid pPB122 to delete *ddcA*. Deletion of *ddcA* was verified via PCR genotyping using oPEB601/602.

PEB643 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, Δ*yneA::loxP*): PEB561 was transformed with editing plasmid pPB122 to delete *ddcA*. Deletion of *ddcA* was verified via PCR genotyping using oPEB601/602.

PEB719 (Δ*ddcP, amyE::*P_{xyl}*-ddcP*Δ*TM*): PEB324 was transformed with plasmid pPB216.

PEB772 (Δ*ctpA, amyE::*P_{xyl}*-ctpA*Δ*TM*): PEB355 was transformed with a PCR product containing the portion of *amyE::*P_{xyl}*-ctpA*Δ*TM* using oPEB370/377, as the plasmid could not be isolated from *E. coli*. Briefly, *ctpA*Δ*TM* was amplified using oPEB911/818, the upstream portion of *amyE* and the P_{xyl} promoter were amplified using oPEB370/383, and the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377. The final PCR product used for transformation of PEB355 was generated using the three preceding PCR products as a template and oPEB370/377.

PEB774 (*ddcP*Δ*PDZ*): PY79 was edited using CRISPR/Cas9 genome editing with plasmid pPB245. Deletion of the sequence encoding the PDZ domain of DdcP was verified via PCR genotyping using oPEB481/482.

PEB776 (*ctpA*Δ*PDZ*): PY79 was edited using CRISPR/Cas9 genome editing with plasmid pPB246. Deletion of the sequence encoding the PDZ domain of CtpA was verified via PCR genotyping using oPEB592/593.

PEB836 (Δ*ddcA*, *amyE::*P_{xyl}*-ddcP*): PEB357 was transformed with pPB108 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB837 (Δ*ddcA*, *amyE::*P_{xyl}*-ctpA*): PEB357 was transformed with pPB184 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB838 (Δ*ddcP,* Δ*ctpA*, *amyE::*P_{xyl}*-ddcA*): PEB555 was transformed with pPB147 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB839 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, *amyE::*P_{xyl}*-ddcP*): PEB639 was transformed with pPB108 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB840 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, *amyE::*P_{xyl}*-ddcA*): PEB639 was transformed with pPB147 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB841 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, *amyE::*P_{xyl}*-ctpA*): PEB639 was transformed with pPB184 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB846 (*amyE::*P_{xyl}*-yneA*): PY79 was transformed with pPB192 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination

was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB848 (Δ*ddcA*, *amyE::P$_{xyl}$-yneA*): PEB357 was transformed with pPB192 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB850 (Δ*ddcP,* Δ*ctpA*, *amyE::P$_{xyl}$-yneA*): PEB555 was transformed with pPB192 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB852 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, *amyE::P$_{xyl}$-yneA*): PEB639 was transformed with pPB192 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB854 (Δ*ddcA*, *amyE::P$_{xyl}$-gfp-ddcA*): PEB357 was transformed with pPB254 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB856 (Δ*ddcA*, *amyE::P$_{xyl}$-ddcA-gfp*): PEB357 was transformed with pPB255 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB876 (*amyE::P$_{xyl}$-gfp-yneA*): PY79 was transformed with pPB257 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB882 (Δ*ddcA*, *amyE::P$_{xyl}$-gfp-yneA*): PEB357 was transformed with pPB257 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB888 (Δ*ddcP,* Δ*ctpA*, *amyE::P$_{xyl}$-gfp-yneA*): PEB555 was transformed with pPB257 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the

absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

PEB894 (Δ*ddcP,* Δ*ctpA*, Δ*ddcA*, *amyE::*P_{xyl}*-gfp-yneA*): PEB639 was transformed with pPB257 digested with two restriction enzymes (KpnI-HF and ScaI-HF). Replacement of *amyE* via double crossover recombination was verified by testing for an inability to utilize starch and for the absence of a spectinomycin resistance cassette present on the portion of the plasmid that is not incorporated.

Plasmid construction

Plasmids were constructed via Gibson assembly (Gibson, 2011) using the PCR amplicons listed below as previously described (Burby et al., 2018).

pPB84: Plasmid pPB84 was constructed via Gibson assembly using four PCR products: 1) the vector pPB41 was amplified using oPEB217/218; 2) Cas9/CRISPR::*uvrB* was amplified using pPB73 as a template with oPEB232/234; 3) the sequence upstream of *uvrAB* for the editing template was amplified using oPEB428/435; and 4) the sequence downstream of *uvrAB* for the editing template was amplified using oPEB436/422. Clones were verified via Sanger sequencing using oPEB227, oPEB253, and oPEB434.

pPB147: Plasmid pPB147 was constructed via Gibson assembly using four PCR products: 1) pPB47 was amplified using oPEB116/117; 2) the upstream portion of *amyE* and the P_{xyl} promoter was amplified using oPEB370/383; 3) the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377; 4) the *ddcA* ORF was amplified using oPEB706/707. Clones were verified via Sanger sequencing using oPEB345, oPEB348, and oPEB708.

pPB192: Plasmid pPB192 was constructed via Gibson assembly using four PCR products: 1) pPB47 was amplified using oPEB116/117; 2) the upstream portion of *amyE* and the P_{xyl} promoter was amplified using oPEB370/383; 3) the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377; 4) the *yneA* ORF was amplified using oPEB854/855. Clones were verified via Sanger sequencing using oPEB345 and oPEB348.

pPB216: The upstream portion of *amyE* and the P_{xyl} promoter were PCR amplified using oPEB370/383. The chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377. Plasmid pPB47 was PCR amplified using oPEB116/117. The ORF of *ddcP* (coding for a.a. 36-341) was PCR amplified using oPEB887/561. These four PCR products were used in a Gibson assembly reaction to generate a plasmid to integrate

*ddcP*ΔTM under the control of P_{xyl} at the *amyE* locus. Clones were verified by Sanger sequencing with oPEB345 and oPEB348.

pPB235: A proto-spacer targeting *ddcP* in the sequence coding for the PDZ domain (oPEB984/985) was ligated to pPB41.

pPB236: A proto-spacer targeting *ctpA* in the sequence coding for the PDZ domain (oPEB988/989) was ligated to pPB41.

pPB245: The upstream and downstream portions of the *ddcP*Δ*PDZ* editing template were PCR amplified using oPEB477/986 and oPEB987/480, respectively. CRISPR/Cas9 was PCR amplified using oPEB232/234 and pPB235 as the template. The pPB41 vector was PCR amplified using oPEB217/218. These four PCR products were used in a Gibson assembly reaction to generate a *ddcP*Δ*PDZ* editing plasmid. Clones were verified by Sanger sequencing with oPEB227, oPEB253, and oPEB483.

pPB246: The upstream and downstream portions of the *ctpA*Δ*PDZ* editing template were PCR amplified using oPEB588/990 and oPEB991/591, respectively. CRISPR/Cas9 was PCR amplified using oPEB232/234 and pPB236 as the template. The pPB41 vector was PCR amplified using oPEB217/218. These four PCR products were used in a Gibson assembly reaction to generate a *ctpA*Δ*PDZ* editing plasmid. Clones were verified by Sanger sequencing with oPEB227, oPEB253, and oPEB594.

pPB254: Plasmid pPB254 was constructed via Gibson assembly using five PCR products: 1) pPB47 was amplified using oPEB116/117; 2) the upstream portion of *amyE* and the P_{xyl} promoter was amplified using oPEB370/383; 3) the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377; 4) *gfp* with a linker was amplified using oPEB998/999; 5) the *ddcA* ORF was amplified using oPEB1000/707. Clones were verified via Sanger sequencing using oPEB345, oPEB348, and oPEB708.

pPB255: Plasmid pPB255 was constructed via Gibson assembly using five PCR products: 1) pPB47 was amplified using oPEB116/117; 2) the upstream portion of *amyE* and the P_{xyl} promoter was amplified using oPEB370/383; 3) the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377; 4) the *ddcA* ORF was amplified using oPEB706/1003; 5) *gfp* with a linker was amplified using oPEB1001/1002. Clones were verified via Sanger sequencing using oPEB345, oPEB348, and oPEB708.

pPB257: Plasmid pPB257 was constructed via Gibson assembly using five PCR products: 1) pPB47 was amplified using oPEB116/117; 2) the upstream portion of *amyE* and the P_{xyl} promoter was amplified using oPEB370/383; 3) the chloramphenicol resistance cassette and the downstream portion of *amyE* were amplified using oPEB557/377; 4) *gfp* with a linker was amplified using oPEB998/1005; 5) the *yneA* ORF was amplified using oPEB1006/855. Clones were verified via Sanger sequencing using oPEB345 and oPEB348.

pPB269: DdcA was amplified with primers oPEB1037/1038. The plasmid pKT25 was amplified with primers oPEB1014/1015. These two PCR products were used in a Gibson assembly reaction to generate a T25-DdcA fusion for expression in BTH101 cells in a bacterial two-hybrid assay. Clones were selected on LB agar containing 50 μg/mL kanamycin and 0.2% glucose. Clones were verified via Sanger sequencing using oPEB708, 1021 and 1022.

Supporting Figures

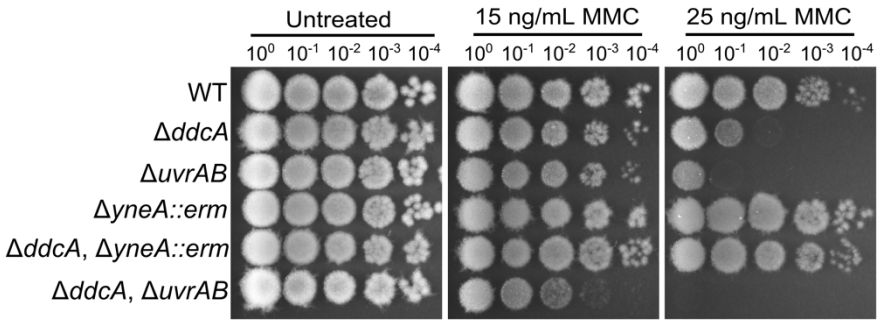

Figure S1. DNA damage sensitivity of the *ddcA* deletion is dependent on the DNA damage checkpoint protein YneA and independent of nucleotide excision repair. A spot titer assay using *B. subtilis* strains WT (PY79), Δ*ddcA* (PEB357), Δ*uvrAB* (PEB309), Δ*yneA::erm* (PEB433), Δ*ddcA* Δ*yneA::erm* (PEB495), and Δ*ddcA* Δ*uvrAB* (PEB497) spotted on the indicated media.

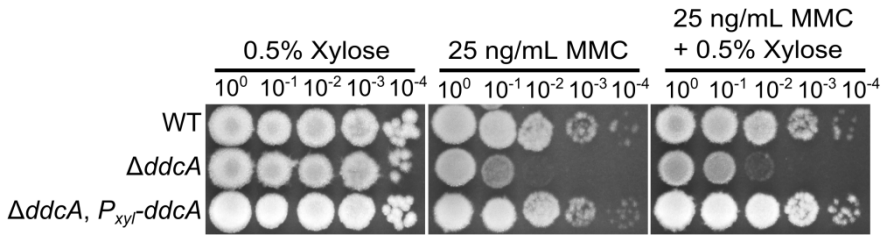

Figure S2. Deletion of *ddcA* can be complemented by ectopic expression using high levels of xylose. A Spot titer assay using WT (PY79), Δ*ddcA* (PEB357), and Δ*ddcA amyE::P*$_{xyl}$*-ddcA* (PEB503) spotted on the indicated media and incubated at 30°C overnight.

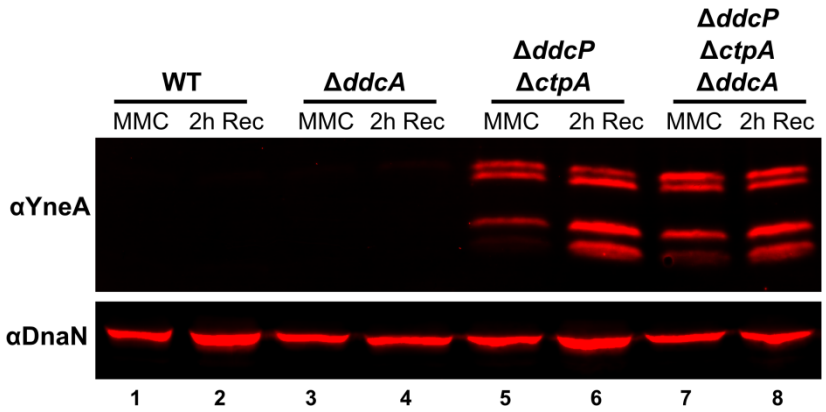

Figure S3. Deletion of *ddcA* does not increase YneA protein levels following MMC treatment and recovery. Western blotting using antisera against YneA (top panel) or DnaN (bottom panel) using whole cell extracts from WT (PY79), Δ*ddcA* (PEB357), Δ*ddcP* Δ*ctpA* (PEB555), Δ*ddcA* Δ*ddcP* Δ*ctpA* (PEB639) after a two-hour treatment with 100 ng/mL MMC (lanes labeled "MMC") or after recovering for two hours from MMC treatment (lanes labeled "2h Rec").

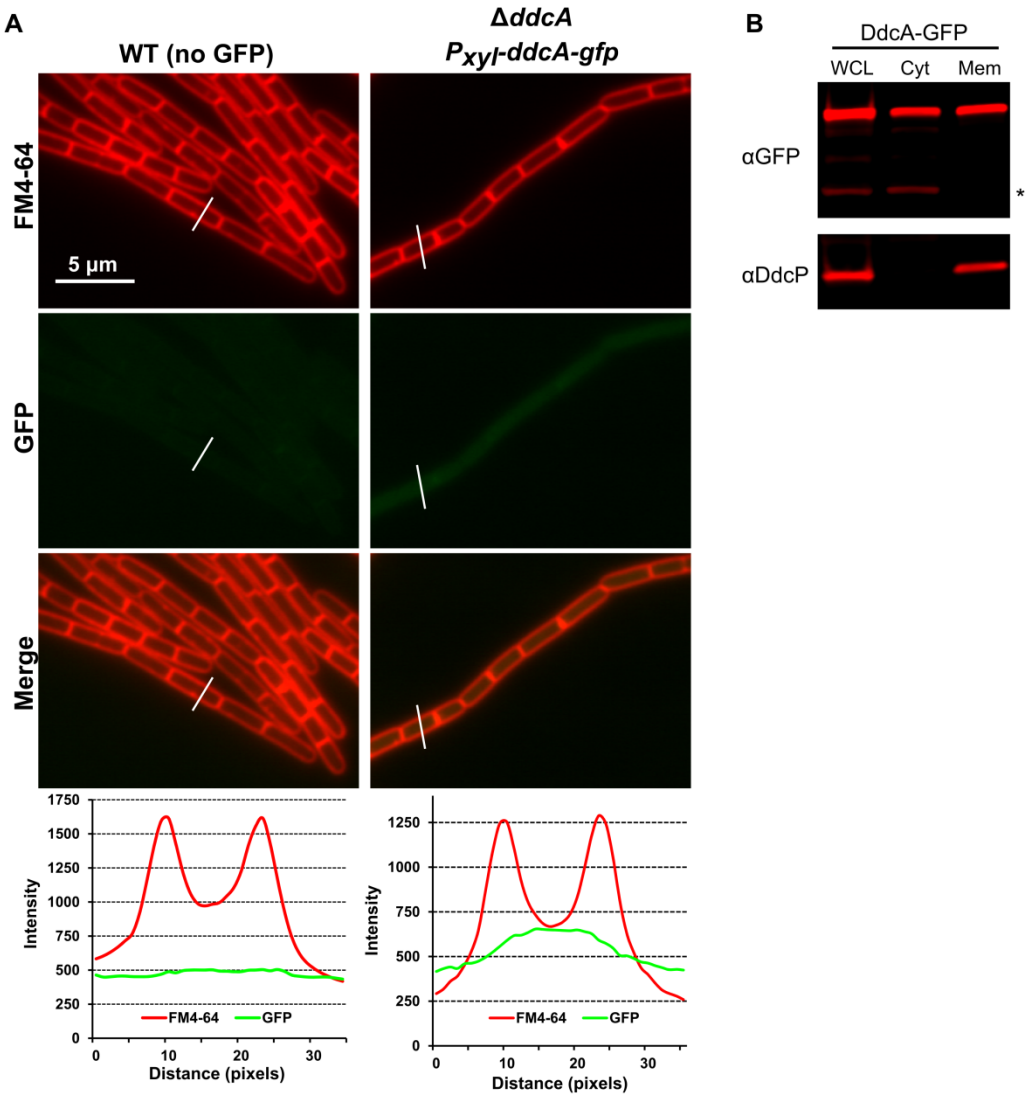

Figure S4. DdcA-GFP is intracellular and found in the cytosolic and membrane fractions.

(A) Micrographs from WT (PY79) and Δ*ddcA amyE::P*$_{xyl}$*-ddcA-gfp* (PEB856) cultures grown in S7$_{50}$ minimal media containing 1% arabinose and 0.05% xylose. Images in red are the membrane stain FM4-64, green are GFP fluorescence and the bottom images are a merge of FM4-64 and GFP fluorescence. The white lines through cells in the images are a representation of the line scans of fluorescence intensity generated in ImageJ and plotted below the micrographs. Scale bar

is 5 µm. **(B)** Western blot of the whole cell lysate (WCL), cytosolic fraction (Cyt), and membrane fraction (Mem) from Δ*ddcA amyE::P*$_{xyl}$*-ddcA-gfp* (PEB856) cell extracts using antisera against GFP (upper panel) or DdcP (lower panel). The asterisk denotes a cross-reacting species detected by the GFP antiserum.

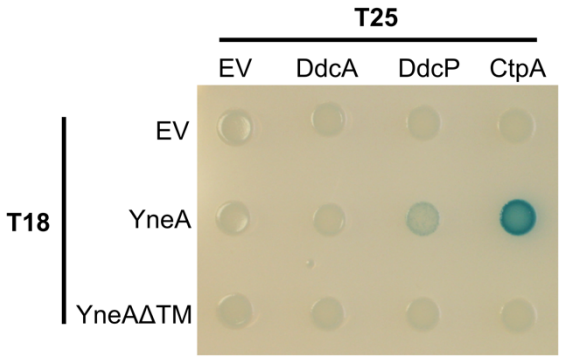

Figure S5. DdcA and YneA do not interact in bacterial two hybrid assay Plasmids containing the indicated T18 (rows) and T25 (columns) fusions were used to co-transform *E. coli* BTH101 cells, which were then spotted onto LB containing X-gal and IPTG.

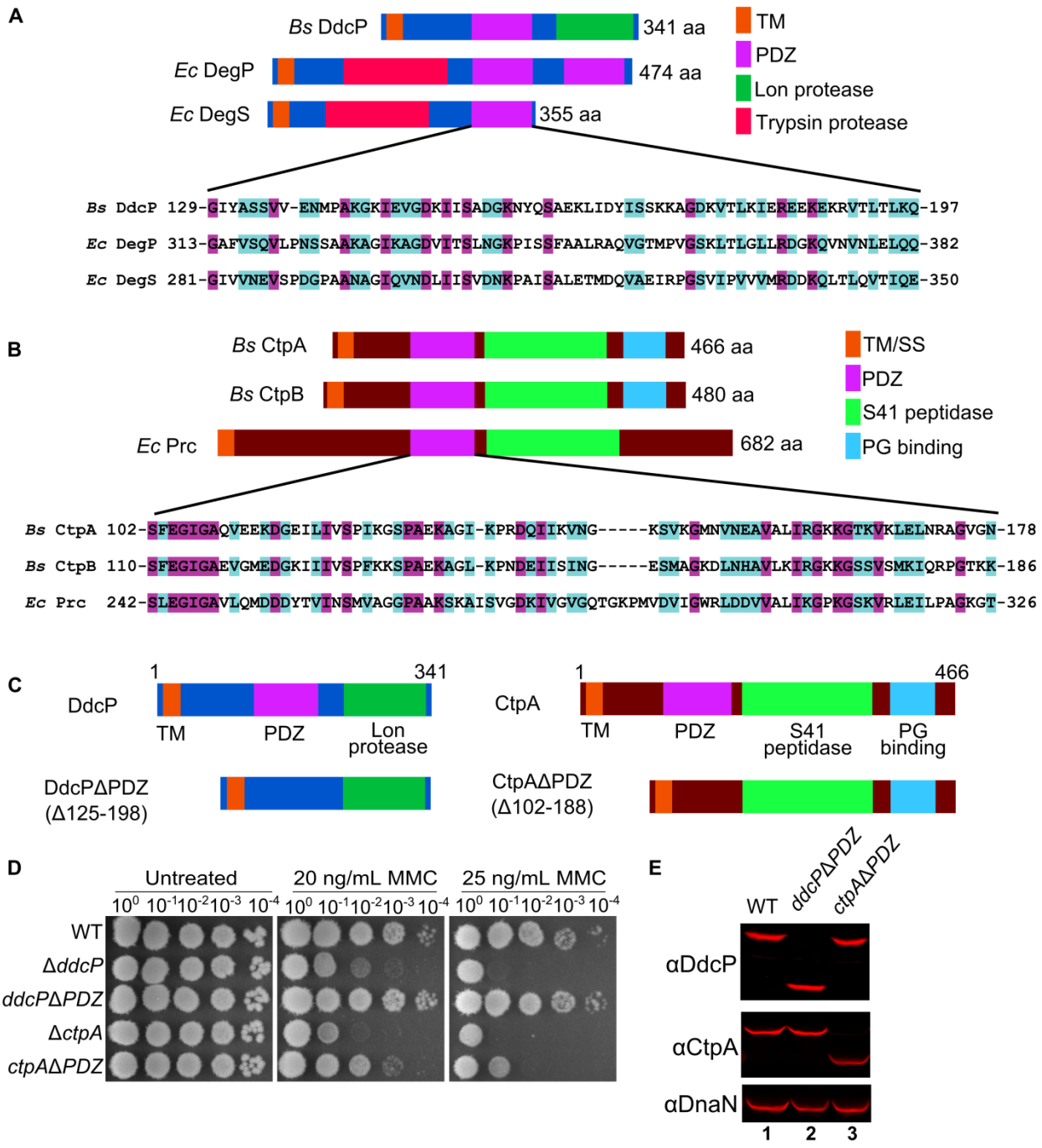

Figure S6. DdcP and CtpA PDZ domains have different functions *in vivo* (A) Alignment of the PDZ domain of DdcP to the PDZ domains of DegP and DegS from *E. coli*. **(B)** Alignment of the PDZ domain of CtpA to the PDZ domains of CtpB from *B. subtilis* and Prc from *E. coli*. **(C)** Schematics of ΔPDZ constructs used in panels B and C. **(D)** Spot titer assay using *B. subtilis*

strains WT (PY79), Δ*ddcP* (PEB324), *ddcP*Δ*PDZ* (PEB774), Δ*ctpA* (PEB355), and *ctpA*Δ*PDZ* (PEB776) media. **(E)** Western blot analysis of WT (PY79), *ddcP*Δ*PDZ* (PEB774), and *ctpA*Δ*PDZ* (PEB776) cell lysates using DdcP, CtpA, and DnaN antiserum.

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