A DNA Damage-Induced, SOS-Independent Checkpoint Regulates Cell Division in Caulobacter crescentus

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A DNA Damage-Induced, SOS-Independent Checkpoint Regulates Cell Division in *Caulobacter crescentus*

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Abstract

Cells must coordinate DNA replication with cell division, especially during episodes of DNA damage. The paradigm for cell division control following DNA damage in bacteria involves the SOS response where cleavage of the transcriptional repressor LexA induces a division inhibitor. However, in *Caulobacter crescentus*, cells lacking the primary SOS-regulated inhibitor, *sidA*, can often still delay division post-damage. Here we identify *didA*, a second cell division inhibitor that is induced by DNA damage, but in an SOS-independent manner. Together, *DidA* and *SidA* inhibit division, such that cells lacking both inhibitors divide prematurely following DNA damage, with lethal consequences. We show that *DidA* does not disrupt assembly of the division machinery and instead binds the essential division protein FtsN to block cytokinesis. Intriguingly, mutations in FtsW and FtsI, which drive the synthesis of septal cell wall material, can suppress the activity of both *SidA* and *DidA*, likely by causing the FtsW/I/N complex to hyperactively initiate cell division. Finally, we identify a transcription factor, *DriD*, that drives the SOS-independent transcription of *didA* following DNA damage.

Introduction

Progress through the cell cycle requires the sequential execution of three fundamental processes: DNA replication, chromosome segregation, and cell division. Maintaining the precise order of these events is crucial to preserving genomic integrity, as any attempt to divide before completing DNA replication or chromosome segregation could result in the scission of DNA and a failure to endow each daughter cell with a complete genome. Coordinating DNA replication and cell division is particularly challenging when cells encounter DNA damaging agents that necessitate lengthy periods of chromosome repair. To ensure the order of cell cycle events and preserve genome integrity, many cells employ checkpoints that actively halt cell cycle progression until DNA damage has been repaired. While checkpoints are prevalent and well characterized in eukaryotes [1], their role and significance in bacteria remains sparsely understood. While checkpoints are prevalent and well characterized in eukaryotes [1], their role and significance in bacteria remains sparsely understood.

In *Caulobacter* the primary SOS-induced division inhibitor is a 29 amino acid inner membrane protein called *SidA* that inhibits division by interacting with the late-arriving division protein FtsW [13]. Although *sidA* is the primary SOS-induced division inhibitor in *Caulobacter*, cells lacking *sidA* can still arrest division when...
grown in the presence of the DNA damaging agent mitomycin C (MMC). An SOS-regulated endonuclease called BapE may indirectly contribute to inhibiting division [14], but we conjectured that Caulobacter encodes another direct cell division inhibitor that is induced by DNA damage but in an SOS-independent manner. Here, we identify such an inhibitor, now named didA. As with sidA, the overexpression of didA in undamaged cells is sufficient to prevent cell division. Cells lacking both inhibitors divide prematurely following DNA damage, leading to a significant viability defect. DidA does not disrupt FtsZ ring formation or divisome assembly and instead likely inhibits division through an interaction with the divisome component FtsN. Intriguingly, point mutations in FtsW and FtsI, which help drive septal cell wall synthesis, suppress the lethality that results from overproducing either SidA or DidA. Our results suggest that these mutations hyperactivate the cell division process and implicate the protein complex FtsW/ I/N in the triggering of cytokinesis. Finally, we identify a transcription factor, DriD, that activates didA expression, thus revealing the basis of a damage-inducible, but SOS-independent pathway in Caulobacter.

Results

Identification of didA, a DNA Damage-Induced, SOS-Independent Cell Division Inhibitor

Our previous work demonstrated that sidA is the primary SOS-induced division inhibitor in Caulobacter. However, many ΔsidA and ΔArcA cells exposed to the DNA damaging agent MMC still become filamentous suggesting that an SOS-independent inhibitor may also prevent division following DNA damage (Figure S1) [13]. To identify candidate inhibitors, we examined global gene expression changes following MMC treatment of a ΔArcA strain, which cannot induce SOS genes. Wild-type and ΔArcA cells were grown to mid-exponential phase in rich medium and exposed to MMC for 30 minutes. RNA was then isolated and compared to mock treated cells on whole genome DNA microarrays (Data S1).

Of the 50 most upregulated genes following MMC treatment in wild-type cells, 44 were recA-dependent, including 31 that are directly regulated by LexA (Figure 1A and S2A) [13,15]. The remaining six damage-regulated genes showed similar induction levels in both wild-type and ΔArcA backgrounds (Figure 1A) and are thus likely controlled by an SOS-independent mechanism. One of these genes, CCNA03212 in the NA1000 (CB15N) genome, encodes a previously uncharacterized 71 amino acid protein with a single predicted transmembrane helix flanked by short cytoplasmic and periplasmic domains (Figure 1B). The open reading frame of CCNA03212 overlaps with the C-terminus of the open reading frame of CC3114, annotated in the closely related strain CB15. In our expression profiling experiments, only those probes lying within the CCNA03212 coding sequence were significantly upregulated in wild-type cells treated with MMC (Figure S2B and S2C), suggesting that the NA1000 annotation is correct. Based on the studies described below, we named this gene didA (for damage-induced cell division inhibitor A).

To confirm that didA encodes a damage-inducible protein, we created a strain in which the chromosomal didA gene was fused to the coding region of the 3×M2 epitope. This C-terminal fusion, DidA-3×M2, was barely detectable in the absence of DNA damage, but was strongly induced following MMC treatment with protein levels increasing nearly 20-fold after 1 hour (Figure 1C). Western blotting indicated a band at the size predicted for DidA-3×M2 (≈11 kDa) and not CC3114-3×M2 (≈25 kDa) indicating that the larger gene product annotated in CB15 is not produced at significant levels in these conditions. To test the SOS-dependence of DidA-3×M2 synthesis following MMC treatment, we examined DidA-3×M2 production in a ΔarcA strain and in a strain harboring lexA(K203A), which encodes a noncleavable form of LexA that blocks the induction of SOS genes. In each case, DidA-3×M2 was slightly elevated in untreated cells, likely due to increased basal levels of damage in the absence of SOS-mediated repair (Figure 1D). Following MMC treatment, DidA-3×M2 was strongly induced in all strains (Figure 1D), consistent with an SOS-independent mode of regulation.

To test whether DidA can inhibit cell division, we fused the didA coding sequence to the vanillate-inducible promoter Pvan and cloned this construct into both low- and medium-copy plasmids. We transformed wild-type cells with each plasmid and then grew cells in the presence of vanillate to induce didA in the absence of a DNA damaging agent. Synthesis of DidA from the low-copy plasmid resulted in mild cellular filamentation and a modest growth defect, while overproduction from the medium-copy plasmid caused a more pronounced division defect with nearly all cells demonstrating severe filamentation after 6 hours (Figure 2A and 2B). Thus, DidA, like SidA, is sufficient to inhibit cell division in the absence of DNA damage.

To assess the level of DidA accumulation during our overproduction experiments, we fused the didA coding sequence to the vanillate-inducible promoter Pvan and cloned this construct into both low- and medium-copy plasmids. We transformed wild-type cells with each plasmid and then grew cells in the presence of vanillate to induce didA in the absence of a DNA damaging agent. Synthesis of DidA from the low-copy plasmid resulted in mild cellular filamentation and a modest growth defect, while overproduction from the medium-copy plasmid caused a more pronounced division defect with nearly all cells demonstrating severe filamentation after 6 hours (Figure 2A and 2B). Thus, DidA, like SidA, is sufficient to inhibit cell division in the absence of DNA damage.
were grown to mid-exponential phase and treated with 1 μm
with 1 lexA(K203A)
cells expressing
and CCNA03212 (didA
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MMC Treatment
help prevent cell division.
MMC exposure (Figure S3B), indicating that the phenotypes observed in Figure 2 are not the result of artificially high DidA levels. Taken together, our results suggest that following DNA damage, DidA accumulates in an SOS-independent fashion to levels. Taken together, our results suggest that following DNA damage, we constructed a strain in which all but the first and last three amino acids of didA were deleted. As with a sidA deletion strain, DidA cells grown on plates containing MMC showed no major viability defect (Figure 3A). However, a strain lacking both sidA and didA showed a pronounced defect, with a nearly 100-fold decrease in plating efficiency (Figure 3A). This decreased viability was rescued by the presence of either inhibitor on a low-copy plasmid (Figure 3B). These results indicate that SidA and DidA are, to some extent, functionally redundant in blocking cell division following MMC-induced DNA damage.

To better understand the DNA damage sensitivity of AsidA:AdidA cells, we used time-lapse microscopy to examine synchronous populations of swarmer cells during growth on agarose pads containing MMC. Wild-type swarmer cells did not divide for ~5 hours on average (Figure 3C), which is significantly longer than the average time to first division of 1.9 hours for wild-type swarmer cells grown on MMC-free pads. On MMC pads, roughly 5% of wild-type cells arrested growth following a cell division event (Figure 3D–3E and Data S2), indicating that division may have been premature or inappropriately executed and was, consequently, lethal. The single deletion strains, AsidA and AdidA, also delayed cell division in the presence of MMC; the average time to division was not significantly different than for wild-type cells. These single deletion strains had 1.5–2 times as many growth arrested cells following division events compared to the wild type, although these defects were apparently insufficient to produce a gross viability defect (Figure 3A and 3D). In contrast to the single mutants, AsidA:AdidA cells lacking both inhibitors divided ~1.25 hours earlier than wild-type (p = 6.9×10−10), and four times as many cells exhibited growth defects following a division event (Figure 3C–3E; Data S2). Taken together, our data suggest that the lethality experienced by AsidA:AdidA cells in the presence of MMC results from an inability to appropriately delay cell division.

DidA Interacts with the Late-Arriving Divisome Component FtsN

We next sought to investigate how DidA disrupts cell division. We first asked whether DidA interferes with cell division directly, through an interaction with the divisome, or indirectly by inducing the SOS regulon or inhibiting the cell cycle regulator CtrA. To investigate the possibility of indirect mechanisms, we isolated RNA from cells overproducing DidA from a medium-copy plasmid for 45 minutes and compared it on DNA microarrays to RNA from similarly treated cells grown in the absence of inducer. No significant gene expression changes were observed in the SOS or CtrA regulons (Data S1B) suggesting that DidA acts post-transcriptionally, and possibly directly, to inhibit cell division.

To further explore how DidA inhibits cell division, we examined its subcellular localization. In predivisional cells, the major components of the cell division machinery are located at mid-cell [2] where they synthesize a septum and drive invagination of the cell envelope. To assess DidA localization, we transformed wild-type cells with a low-copy plasmid harboring an M2-yfp-didA fusion under the control of a xylose-inducible promoter. After induction for 3 hours, cells became filamentous indicating that the YFP-DidA fusion inhibits cell division (Figure 4A). Notably, YFP-DidA foci were frequently observed at pinch sites near mid-cell (Figure 4A) placing it in close proximity to the cell division machinery. Further, fractionation of cells overproducing 3×M2-DidA indicated that DidA is strongly enriched in the membrane where many of the middle- and late-arriving cell division components also reside (Figure 4B). These data are consistent with a model whereby DidA inhibits division through an interaction with a component of the divisome.
To test for interactions of DidA with the known set of critical Caulobacter cell division components [2], we performed a bacterial two-hybrid analysis as used previously with SidA [13,16]. Briefly, proteins were fused to either the T18 or T25 subunit of adenylate cyclase and co-expressed in E. coli; a protein-protein interaction reconstitutes adenylate cyclase and drives synthesis of cyclic-AMP, causing colonies to appear red on MacConkey agar plates. When expressed from the low-copy plasmid pKT25, a T25-DidA fusion interacted almost exclusively with the late-arriving cell division protein fusion T18-FtsN (Figures 4C and S4A). Identical results were obtained in the reciprocal orientation, with a T18-DidA fusion on the high-copy plasmid pUT18C and individual division proteins produced from pKT25 (Figure S4B). SidA, whose primary target is likely FtsW, also interacts, to some extent, with FtsN (Figure 4C) [13]. In sum, our data suggest that DidA is an integral membrane protein that localizes to mid-cell where it may disrupt cell division through an interaction with FtsN.

FtsN is among the last cell division proteins to arrive at mid-cell prior to cytokinesis. Although its precise function is unknown, FtsN interacts with multiple division proteins and may help stabilize the assembled divisome [16–18]. To ask whether DidA destabilizes or blocks assembly of the divisome, we examined the localization of early- and late-arriving division proteins during DidA overproduction. Cells producing fluorescently tagged FtsZ, FtsW, FtsI, or FtsN were transformed with a plasmid for overexpressing didA and then grown in the presence of vanillate to induce DidA synthesis. After 4.5 hours of induction, cells expressing ftsZ-yfp, venus-ftsW, or gfp-ftsI were inhibited for cell division, but 89%, 95%, and 85% of cells, respectively, contained fluorescent foci at or near visible pinch sites (Figure 4D). These results indicate that DidA likely does not disrupt the localization of cell division proteins or drive the disassembly of division protein complexes. Additionally, we noted that many cells displayed multiple foci of the FtsZ, FtsW, or FtsI fluorescent fusions suggesting that DidA also does not prevent the formation of new division assemblies.

Intriguingly, cells expressing gfp-ftsN were noticeably shorter (12.6 ± 0.65 μm standard error of the mean [SEM]) and more pinched than those expressing ftsZ-yfp, venus-ftsW, or gfp-ftsI (22.8 ± 0.79, 24.7 ± 1.05, 26.1 ± 0.74 μm, respectively) (Figure 4D). Further, cells expressing gfp-ftsN robustly formed colonies despite DidA overproduction, in contrast to cells expressing the other fluorescent fusions (Figure 4E), indicating that gfp-ftsN functions as a DidA suppressor, possibly by decreasing its affinity for DidA or by stabilizing FtsN and thereby increasing FtsN levels. In either case, these data further support a model in which DidA interacts with FtsN to block cell division, but without disrupting assembly of an intact divisome.

We next sought to determine whether point mutations in FtsN can also suppress the lethality of overproducing DidA. We first constructed a low-copy plasmid on which $\delta M2$-didA was transcribed from the IPTG-inducible promoter $P_{lac}$. We then used mutagenic PCR to create a library of ftsN mutants containing, on average, one nucleotide substitution per coding sequence; these ftsN mutants were cloned into a medium-copy plasmid with expression driven by $P_{xyl}$. The didA expression vector and ftsN plasmid library were co-transformed into an ftsN deletion strain in which the only chromosomal copy of ftsN is transcribed from the $P_{van}$ locus [19]. Cells were plated in the presence of IPTG to induce $3\times M2$-DidA,
but without vanillate such that only plasmid-produced, mutant FtsN accumulated. From ~168,000 cells plated, two candidate ftsN suppressors were isolated that suppressed the lethality of overproducing DidA. Plasmid sequencing indicated that one clone contained a single mutation, *ftsN(L202P)*, while the other contained two mutations, *ftsN(P156S)* and *ftsN(F252L)*.

Each mutation was introduced into an otherwise wild-type chromosome and tested for its ability to suppress 36M2-DidA overproduction. Only those cells harboring the *ftsN(L202P)* or *ftsN(F252L)* mutation maintained 36M2-DidA suppression (Figure 5A and 5B), indicating that *ftsN(P156S)* was likely a passenger mutation with *ftsN(F252L)*. Intriguingly, both *bONA fide* suppressor mutations reside within the periplasmic, C-terminal “SPOR” domain of FtsN, which may bind peptidoglycan structures within the actively dividing, septal cell wall [19–21]. To further explore the regions of FtsN that bind DidA, we tested a series of FtsN truncations and chimeras in the bacterial two-hybrid system (Figure 5C). T25-DidA still interacted with an FtsN construct whose cytoplasmic and transmembrane domains were replaced with the transmembrane domain of the *E. coli* permease MalF, but not with a MalF fusion to the divisome component FtsA. In contrast, the DidA-FtsN interaction was significantly weakened when FtsN constructs lacked either its entire periplasmic portion or the periplasmic SPOR domain alone. We also noted that DidA still interacted robustly with an FtsN construct in which the only known essential domain, located within the periplasmic linker region and denoted “H1” [19], was replaced with an unstructured region of the *Caulobacter* protein SpmX. Collectively, these results suggest that DidA binds the periplasmic SPOR domain of FtsN where the suppressor mutations L202P and F252L.

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Figure 3. Cells lacking *sidA* and *didA* cannot properly regulate cell division following DNA damage. (A) Wild-type, *ΔsidA*, *ΔdidA*, and *ΔsidAΔdidA* cells were grown to mid-exponential phase and plated in 10-fold dilutions on rich media with or without 0.35 μg/ml MMC. (B) Wild-type and *AsidAΔdidA* cells carrying an empty plasmid, and *ΔsidAΔdidA* cells carrying a plasmid with either *sidA* or *didA* driven by its native promoter were plated as in (A). (C–E) Synchronous populations of swarmer cells from the strains in (A) were placed on agarose pads containing rich media and MMC and imaged for 8 hours by time-lapse microscopy. (C) The time to first mid-cell division and (D) the percentage of cells that stopped growing following division relative to the wild type are shown (for criteria on calling divisions and growth cessation, see Text S1). The data in (C) are representative of biological duplicates. The data in (D) are averaged from biological duplicates. Asterisks represent a statistically significant (*p* < 0.01) difference relative to the wild type. Error bars represent standard error of the mean (SEM). (E) Representative fields of wild-type and *ΔsidAΔdidA* swarmer cells grown on pads containing MMC at the time points indicated in hours. Black arrows indicate cells that divided. Gray arrows indicate cells arrested for growth following division. Bar, 2 μm. doi:10.1371/journal.pbio.1001977.g003
reside. Moreover, we found that, when introduced into T18-FtsN, each suppressor mutation strongly reduced the interaction with DidA compared to wild-type FtsN or FtsN(P156S) which, as noted, does not suppress DidA lethality (Figure 5C). Importantly, each of the FtsN mutants tested interacted with FtsW as well as the wild-type FtsN did, indicating that the mutants were properly expressed and folded. In summary, our results suggest that DidA binds the SPOR domain of the late-arriving divisome component FtsN, and the substitutions L202P and F252L in this domain suppress the lethality of overproducing DidA by reducing its affinity for FtsN.

Mutations in ftsW Can Suppress the Division Inhibition Caused by Either SidA or DidA

To further explore the mechanism by which DidA inhibits division, we also screened for spontaneous mutations that suppress the lethality of overproducing DidA. Wild-type cells carrying a medium-copy plasmid expressing 3×M2-didA from the P\text{van} promoter on a low-copy plasmid. Cells were grown to mid-exponential phase in rich media with glucose and then shifted to xylose. At the times indicated, cells were imaged by phase and epifluorescent microscopy. In the fluorescent micrographs, cell boundaries were added after imaging. (B) Subcellular fractionation of cells overexpressing 3×M2-didA from the P\text{van} promoter on a medium-copy plasmid for 1.5 hours and expressing the transmembrane protein cckA-gfp from P\text{cckA} on the chromosome. Samples were fractionated into soluble (S) and membrane (M) fractions and analyzed by Western blot. The membrane was cut into three pieces, indicated by dashed lines, and probed with antibodies specific for the GFP, CtrA, or M2 epitope. (C) Bacterial two-hybrid analysis of interactions between T25-DidA and cell division proteins fused to T18, as indicated. The FtsI\text{DC} construct lacking the C-terminal catalytic domain previously showed interactions with FtsW and FtsN as expected, unlike the full-length version of FtsI [13]. The interacting pair T18-M2-SidA and T25-FtsN was included for comparison. E. coli strains harboring each pair of fusions were plated on LB, and colonies were restruck on MacConkey plates containing maltose. Red streaks indicate positive interactions. −/− indicates empty vectors negative control, +/+ indicates the zip/zip fusions used as a positive control. (D) Subcellular localization of FtsZ, FtsW, FtsI, and FtsN were examined in strains expressing ftsZ-yfp from the chromosomal P\text{van} promoter, or venus-ftsW, gfp-ftsI or gfp-ftsN from its native chromosomal locus. Each strain was transformed with a medium-copy plasmid expressing didA from the P\text{van} promoter. Strains were grown to mid-exponential phase and samples imaged by phase and epifluorescent microscopy after addition of vanillate for 4.5 hours. In the fluorescent images, cell outlines were drawn based on the phase micrographs. Bar, 2 μm. (E) Strains from (D) were grown to mid-exponential phase and 10-fold serial dilutions were plated on rich media supplemented with vanillate to induce didA expression.

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suppressor mutations. From roughly $3 \times 10^7$ plated cells, 34 suppressors were identified, although only one strain retained high levels of functional $36\text{M2-DidA}$. Whole genome resequencing identified a putative suppressor mutation in $\text{ftsW}$, which would produce the substitution A246T in the predicted large periplasmic loop of FtsW (Figure 5A). This mutation was created de novo in a wild-type background and confirmed to suppress the lethality of overproducing DidA (Figure 5B). As noted, no interactions between DidA and FtsW were observed in our two-hybrid analysis. This could be a false negative; alternatively, FtsW(A246T) may suppress DidA overproduction by promoting an activity of FtsW rather than by preventing binding of the inhibitor.

Intriguingly, we had previously found other mutations in $\text{ftsW}$ that suppress the lethality of overproducing SidA [13]. We therefore reasoned that SidA and DidA may function similarly to inhibit cell division. To explore this possibility, we asked whether the previously identified suppressors of SidA overproduction could also suppress the activity of DidA, or vice versa (Figure 5C and S5) [13]. The other mutations showed varying abilities to suppress the lethality associated with overproducing either inhibitor. In particular, the strains producing FtsW(F145L) or FtsW(A246T) showed robust suppression of both inhibitors.

The ability of these single substitutions, F145L and A246T, to suppress the lethality of overproducing either SidA or DidA could indicate that the inhibitors share a binding site within FtsW that is disrupted by the suppressor mutations. However, this is unlikely given that (1) DidA binds FtsN, but not FtsW, in our bacterial two-hybrid system, (2) DidA-YFP still localizes to the septum in cells producing FtsW(A246T) (Figure S5A), and (3) M2-SidA binds to FtsW(A246T) to the same extent as it does to wild-type FtsW (Figure S5B). Instead, we hypothesized that the subcomplex of late-arriving division components FtsW, FtsI, and FtsN could exist in one of two states: an active state that promotes constriction of the septum and cell division, and an inactive state that is promoted or stabilized by SidA and DidA. In this model, the suppressor mutations in $\text{ftsW}$ and $\text{ftsI}$ promote the active state and thus enable cell division even in the presence of SidA and DidA.

**SidA and DidA Suppressor Mutations Drive Hyperactive Cell Division**

If the FtsW(F145L) and FtsW(A246T) mutations promote an active state of a subcomplex of cell division proteins, then cells harboring these mutations, but not producing SidA or DidA, may
attempt division earlier than wild-type cells, even in the absence of DNA damage. To explore this possibility, we grew strains harboring one of the suppressor mutations in ftsW, ftsI, or ftsN into mid-exponential phase in rich medium and measured cell lengths in a large population of cells. Indeed, several of the suppressor mutations resulted in cells that were significantly shorter on average than wild-type cells even though their growth rates were not substantially different (Figures 6A, 6B, and S6A–S6C). For ftsW(A246T), we verified that all cell types were shorter, indicating that the mutant strains are not trivially enriched for swarmers (Figure S6B). The degree of shortening roughly correlated with the ability to suppress both SidA and Dida activity, as cells harboring the mutations ftsW(A246T), ftsW(F145L), and ftsI(I45V) that were best able to suppress both SidA and Dida were also the shortest. Conversely, mutations that only suppressed the activity of one inhibitor were typically not shorter than wild-type. We found that AsidaAdida cells were also not shorter than wild-type cells. Taken together, these results are consistent with a model in which suppressors exhibiting short cell phenotypes harbor gain-of-activity mutations rather than simply being defective for SidA or Dida binding.

Given that the ftsW(A246T) mutation renders cells insensitive to SidA and Dida, this suppressor strain should also divide earlier than wild-type cells in the presence of MMC like the AsidaAdida deletion strain. To test this prediction, we grew populations of wild-type and ftsW(A246T) cells on agarose pads containing MMC and measured the time to first division by time-lapse microscopy. The ftsW(A246T) cells divided an average of 35 minutes earlier than wild-type cells and showed a 5-fold increase in the fraction of cells that stopped growing following a division event (Figure S7A and S7B). Accordingly, ftsW(A246T) cells showed a similar sensitivity on MMC plates as observed with the AsidaAdida strain (Figure 6C).

Although the ftsW(A246T) and AsidaAdida strains behave similarly in the presence of MMC, only the ftsW(A246T) strain exhibited a shortened cell phenotype when grown without MMC (Figure 6A and 6B). The ftsW(A246T) cells grew at approximately the same rate as wild-type cells in the absence of MMC; these cells are born shorter than wild-type cells, but also divide when shorter than wild-type cells resulting in nearly identical division cycle times (Figure S6B–S6D). The short cell phenotype of this strain in the absence of MMC suggested that FtsW(A246T) harbors increased cell division activity, and has a propensity to divide early, without being defective for SidA or DidA binding.

We also noticed that the ftsW(A246T) strain grew more slowly than wild-type or ftsW(A246T) cells in liquid cultures (Figure S6C). Because FtsW and FtsI participate in septal cell wall synthesis, we suspected that this growth phenotype may result from premature or misregulated cell division events that compromise cell wall integrity. To test this possibility, we stained wild-type, AsidaAdida, ftsW(A246T), and ftsW(A246T) cells with propidium iodide (PI), a dye that binds nucleic acids, but only if the cell envelope is compromised (Figure 6D). Whereas wild-type, AsidaAdida, and ftsW(A246T) cells were rarely (0.1%–0.3% of cells) stained by PI, 2.6% of ftsW*(*) cells were PI-positive. Given these results, we also tested whether the ftsW(A246T) and ftsW(+) cells were more sensitive than wild-type when treated with cephalexin, which interferes with septal cell wall synthesis by blocking the transpeptidase activity of FtsI. Cephalexin does not directly cause DNA damage, and cells treated with cephalexin showed no noticeable induction of sidA or didA (Figure S8). It was thus not surprising that AsidaAdida cells showed no growth defect compared to wild-type when grown on plates containing a low dose of cephalexin that does not significantly perturb growth or division in wild-type cells (Figure 6C and 6D). In contrast, the ftsW(A246T) and ftsW(+) strains each exhibited cephalexin sensitivity, particularly ftsW(+) (Figure 6C). When grown as liquid cultures with cephalexin, the ftsW(A246T) and ftsW(+) strains had 18- and 40-fold, respectively, more PI-positive cells than wild-type (Figures 6D and S9). By contrast, there was not a similar enrichment of PI-positive cells in the ftsW(A246T) and ftsW(+) strains following an MMC treatment. Furthermore, while the average lengths of cells from the suppressor strains were decreased relative to wild type in MMC, likely due to premature divisions, they were longer in cephalexin, indicating a decreased ability to divide (Figure S9).

In sum, cells harboring the mutation ftsW(A246T), either alone or in combination with ftsI(I45V) and ftsW(F145L), exhibit cell wall defects and are more sensitive to a cell wall synthesis inhibitor. Importantly, cells lacking sidA and didA do not exhibit these same cell wall defects. These results are consistent with a model in which the mutations identified in ftsW and ftsI do not suppress SidA and Dida by simply preventing the binding of these inhibitors, but instead affect septal cell wall synthesis and increase the propensity of cells to initiate cell division.

sidA and didA Are Differentially Regulated

Our identification of didA indicates that Caulobacter cells have an SOS-independent mechanism for sensing and responding to DNA damage. To explore this alternative, damage-inducible pathway, we first asked whether didA is induced specifically by DNA damage or more generally by cellular stress. Cells harboring a didA-3×M2 fusion at the native didA locus were treated with a variety of stresses, but the only conditions leading to a significant induction of didA were DNA damaging agents (Figure S10).

To further examine didA induction and compare it to sidA induction, we transformed wild-type cells with plasmids harboring a transcriptional fusion of egfp to either the sidA or didA promoter and then treated each strain with (i) MMC, an alkylating agent that forms single-stranded DNA adducts and double-stranded cross-links, (ii) hydroxyurea, which depletes the dNTP pool by inhibiting ribonucleotide reductase and stalls replication forks, thereby mimicking a consequence of DNA damage, or (iii) zeocin, which directly cleaves DNA, creating double-strand breaks. Western blots for GFP indicated that MMC strongly induced both sidA and didA (Figure 7A). In contrast, hydroxyurea drove induction of P_{sidA}, but not P_{didA}, even at high doses. Conversely, zeocin strongly induced P_{didA}, but only weakly induced P_{sidA}. These data indicate that the SOS-independent induction of didA involves a signal or DNA structure that is distinct from the ssDNA-RecA-dependent induction of sidA. In particular, the strong induction of P_{didA} by zeocin suggests that the signal may be a DNA structure associated with the presence or repair of double strand breaks, which also arise following MMC exposure [22].
Identification of driD, an SOS-Independent, DNA Damage-Induced Transcription Factor

We devised a genetic screen to identify factors involved in didA induction. In a ΔdidA background, we fused the didA promoter to lacZ and integrated this reporter construct at the hfaB locus, a region of low transcription. When grown in the presence of X-gal, colonies with high P_didA activity should express lacZ and appear blue while those with low P_didA activity should appear white. We mutagenized this strain using a Tn5 transposon and screened for mutants on X-gal plates containing MMC. We chose a dose of MMC low enough to allow colony formation, but high enough to induce didA induction resulting in blue colonies. We screened ~26,000 colonies and isolated nine white colonies; five of these colonies had Tn5 insertions in the P_didA-lacZ reporter while the remaining four contained insertions in the coding region of CCNA_01151 (Figure 7B). This gene is annotated as a DeoR-family transcriptional regulator and is predicted to encode an N-terminal DNA-binding domain with a C-terminal ligand-binding domain (“WYL domain,” Pfam domain 13280). Each of the four insertions in CCNA_01151 was unique with one occurring in the DNA-binding domain and the other three in the C-terminal WYL domain. We named CCNA_01151 driD (for DeoR inducer of didA).

To confirm that DriD induces didA, we constructed a strain in which all of driD except the first three and last ten amino acids were deleted. We then transformed wild-type, ΔdriD, and ΔrecA cells with low-copy plasmids harboring P_sidA-egfp or P_didA-egfp reporters and monitored the inducibility of each promoter following MMC or zeocin treatment by Western blotting with α-GFP (Figure 7C). As expected, sidA induction by either DNA damaging agent requires the SOS regulator gene recA but is unaffected in cells lacking driD. In contrast, didA induction occurs in ΔrecA cells but not in cells lacking driD. These results confirm the SOS-independent inducibility of didA and indicate that driD is required for didA induction. We also tested whether the driD deletion behaves like a didA deletion with respect to MMC sensitivity (Figure 7D). Indeed, cells lacking both sidA and driD exhibited a roughly 100-fold reduction in viability when grown on MMC plates, compared to the wild type and strains lacking either sidA or driD. A nearly identical defect was observed when combining sidA and didA deletions, further supporting a model whereby DriD drives didA induction.

We next sought to complement our driD deletion by introducing low-copy plasmids containing P_driD fused to wild-type

Figure 6. Mutations that suppress sidA and didA overexpression likely hyperactivate cell division. (A) The strains indicated were grown to mid-exponential phase in rich media and imaged by phase microscopy. Bar, 2 μm. (B) Each strain indicated was grown to mid-exponential phase and average cell length, relative to wild-type, was calculated (all n>440). Error bars represent standard error of the mean (SEM), and asterisks indicate p<0.01 (*) or p<0.0001 (**). The strain denoted ftsW**I* combines the mutations ftsW(F145L, A246T) and ftsI(I45V). Separate graphs are shown for cell length measurements made on different days. For raw data, see Data S3. (C) Wild-type, ΔsidAΔdidA, ftsW(A246T), and ftsW**I* cells were grown to mid-exponential phase and plated in 10-fold dilutions on rich media containing no additives, 0.35 μg/ml MMC or 6 μg/ml cephalexin. (D) The strains from (C) were grown to mid-exponential phase in rich media and treated with MMC or cephalexin at the concentrations in (C) for 6 hours. PI at 5 μM was added 1.5 hours before imaging. Cells were imaged by phase and fluorescence microscopy; cell lengths and percentage of PI+ cells are shown by bar graphs. For raw data, see Data S3.

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driD or a copy of driD encoding an N- or C-terminal fusion to the 3×M2 epitope; each strain also harbored a chromosomal didA-3×M2 reporter to assess DriD activity. Whereas cells carrying an empty vector were unable to induce didA when treated with zeocin, cells with wild-type or either tagged version of driD were able to induce didA (Figure 7E, bottom panel). Additionally, we noted that the levels of both 3×M2-tagged DriD constructs remained unchanged following zeocin treatment (Figure 7E, top panel) indicating that DriD activity is regulated post-translationally.

Finally, to determine whether DriD directly activates didA, we assessed DriD occupancy at PdidA using chromatin immunoprecipitation (ChIP) followed by quantitative PCR. Cells expressing driD or driD-3×M2 from a plasmid as the only copy of driD were treated with 15 μg/ml zeocin for 45 minutes. DriD was immunoprecipitated with an α-FLAG/M2 antibody and promoter occupancy was analyzed by quantitative PCR using primers specific for PdidA. Fold-enrichment values were normalized relative to the enrichment of a region within the coding sequence of ruvA. For raw data, see Data S3.

**Discussion**

**SOS-Independent Regulation of the DNA Damage Response**

During episodes of DNA damage, cells often use checkpoint systems to transiently inhibit the cell cycle and prevent cell division [23]. In bacteria, the regulatory paradigm for responding to DNA damage has long been the *E. coli* SOS system in which cleavage of the repressor LexA drives the transcription of DNA repair genes and the cell division inhibitor sulA [8,9,24]. SOS-induced division inhibitors have subsequently been identified in a range of other bacteria, including sulA homologs in γ-proteobacteria and the unrelated genes yneA, divS, chiz, and sidA in various other species [10–13,25]. Although these SOS-dependent regulators are often assumed to be the primary, or even sole, mechanism for inhibiting division post-damage, there have been hints of SOS-independent division regulation. For instance, in *E. coli*, *Bacillus subtilis*, and
**Caulobacter**, cells lacking their SOS-induced inhibitors or unable to induce an SOS response can still become filamentous following DNA damage indicating an alternative means of blocking cell division [26–30]. However, to the best of our knowledge, no damage-induced, SOS-independent division regulators have been previously documented. Here, we identified *didA* in *Caulobacter* as one such regulator.

How do *Caulobacter* cells recognize and respond to DNA damage to induce *didA* if not through the canonical derepression of SOS genes? DriD is a direct transcriptional activator of *didA*, but how does DriD sense DNA damage? One possibility is that DriD somehow senses the accumulation of the SOS signal ssDNA, which stimulates RecA to trigger the autocatalytic cleavage of LexA [31–33]. Another protein, such as the RecA homolog RadA, could also recognize ssDNA, but ultimately activate DriD. However, this scenario is unlikely given the differential induction of *sidA* and *didA* following exposure to DNA damaging agents with distinct mechanisms. Alternatively, a DNA damage sensor unrelated to RecA could recognize a distinct type of DNA damage or DNA structure. For instance, the strong induction of *didA* following zeocin exposure could indicate that the *didA* induction machinery recognizes double-strand breaks. In *B. subtilis*, the diadenylate cyclase DisA monitors genome integrity and may recognize branched DNA structures that arise during the recombination-based repair of double-strand breaks [34]. When paused at such DNA structures, DisA is prevented from synthesizing cyclic-di-AMP (c-di-AMP), a diffusible molecule required for the activation of the transcription factor Spo0A, thereby coupling DNA damage with transcription [34–36]. It remains unclear precisely how c-di-AMP affects Spo0A activity in *B. subtilis* and whether a c-di-AMP-based response to DNA damage extends to other organisms. Nonetheless, *didA* transcription could follow a similar regulatory strategy that relies on c-di-AMP, or another damage-regulated second messenger. This is a particularly attractive hypothesis since DriD, annotated as a DeoR-family transcription factor has a C-terminal domain predicted to bind a small molecule. Additionally, we found that DriD levels did not change following zeocin treatment, but occupancy and activation of the *P_didA* promoter by DriD increased significantly. This finding suggests that DriD activity is post-translationally regulated in a DNA damage-dependent manner, so identification of the putative DriD ligand will be a critical next step.

**The Execution and Regulation of Cell Division**

Many cell division inhibitors, including *E. coli* SulA, block cell division by disrupting FtsZ polymerization. FtsZ is an effective target as it recruits most other cell division proteins. However, neither DidA nor SidA affect the assembly of FtsZ rings in *Caulobacter* or stimulate Z-ring disassembly, and neither inhibitor prevents the assembly of downstream divisional components. Instead, these inhibitors appear to block cell division by targeting FtsW, FtsI, and FtsN within the assembled divisome. Bacterial two-hybrid studies indicated that DidA interacts with FtsN. Additionally, several point mutations in *ftsN* diminish the interaction with DidA and suppress the effects of overproducing DidA, supporting a model in which DidA inhibits cell division by binding directly to FtsN, although it remains formally possible that an *E. coli* divisome protein bridges DidA and FtsN in the two-hybrid analysis. SidA interacts with FtsW and FtsN in the bacterial two-hybrid system, and the lethality of overproducing SidA can be suppressed by mutations in either FtsW or FtsI [13]. Although DidA and SidA bind different proteins, these two inhibitors likely inhibit division in similar ways as two mutations in *ftsW*, and one in *ftsI*, can suppress the effects of overproducing either SidA or DidA.

FtsW, FtsI, and FtsN are among the last essential proteins recruited to the cytokinetic ring. These proteins physically interact with each other and likely form a subcomplex within the divisome that drives the synthesis and remodeling of the septal cell wall [2,37–39]. Although its precise biochemical function is unknown, FtsW somehow contributes to septal cell wall synthesis, as does FtsI, which harbors peptidoglycan transpeptidase activity [40,41]. The function of FtsN is also unclear, although in *Caulobacter* its essential activity is located within a periplasmic linker domain [19].

In both *Caulobacter* and *E. coli*, FtsN recruits proteins involved in cell wall remodeling to the division site [42–46], and *E. coli* FtsN has been suggested to stimulate the transpeptidase activity of PBPIB and could act similarly on FtsI [47].

How do single mutations in FtsW and FtsI prevent the inhibition of cell division by both SidA and DidA? One possibility is that these mutations reduce the affinities of SidA and DidA for their division protein targets. However, SidA binding to FtsW was unaffected by the A246T mutation and DidA binds FtsN, not FtsW or FtsI, in our bacterial two-hybrid system. Another possibility is that SidA and DidA block the receptor of multiple peptidoglycan remodeling factors, thereby preventing division. However, given that the inhibitory activity of both SidA and DidA can be suppressed by mutations in FtsW and FtsI, this model seems unlikely.

Instead, we favor a model in which the FtsW/FtsI/FtsN subcomplex exists in two states: an inactive state that is promoted by SidA or DidA, and an active state that drives septal peptidoglycan synthesis and cytokinesis (Figure 8A). We propose that the mutations that suppress both SidA and DidA, such as FtsW(A246T), may lock FtsW/FtsI/FtsN in the active state allowing cells to bypass the block in division normally caused by an accumulation of these inhibitors. On their own, these suppressor mutations cause cells to initiate division hyperactively. In support of this model, cells with the suppressing mutations were reproducibly shorter than wide-type cells (Figure 6A and 6B), likely because they divide at a slightly earlier stage of the cell cycle. Additionally, cells producing FtsW(A246T) or both FtsW(F145L, A246T) and FtsI(145V) were sensitive to cephalaxin, a cell wall synthesis inhibitor, and exhibited compromised cell envelope integrity. Importantly, ΔsidAΔdidA cells did not exhibit increased sensitivity to cephalaxin, further supporting the notion that these mutations in FtsW and FtsI do not simply prevent SidA and DidA binding, but rather increase a cell wall synthesis activity.

Taken together, our results suggest that the DNA damage-induced division inhibitors in *Caulobacter* target the FtsW/FtsI/FtsN subcomplex to block constriction of the division machinery and cell envelope. Precisely how SidA and DidA block division is not yet clear, in part because the execution of cytokinesis remains poorly characterized at a molecular level. The synthesis of septal cell wall material could provide the force and directionality for cellular constriction, with FtsZ required mainly for mid-cell positioning of division proteins. This model is supported by recent data showing that FtsZ often dissociates from the divisome before compartmentalization occurs, indicating that cell wall synthesis may provide the constrictive force for cell division [48]. In such a case, SidA and DidA could prevent division by blocking a critical or rate-limiting peptidoglycan modifying activity of the FtsW/
ftsW/FtsN subcomplex. As noted, the suppressor mutants in ftsW such as A246T that bypass both SidA and DidA are, on their own, prone to disruption of cell envelope integrity. Their sensitivity to cephalaxin could result from certain cell wall synthesis or remodeling activities continuing without concurrent activation of the FtsI transpeptidase domain. As an alternative to this cell wall-centric model for cytokinesis, GTP hydrolysis by the FtsZ ring may provide the energy for, and directionality of, constriction, effectively pulling the rest of the cytokinetic ring along with it [49]. Assembly or activity of the FtsW/FtsI/FtsN subcomplex could somehow trigger FtsZ constriction, and the inhibitors SidA and DidA may block this step of division. Finally, it is possible that Z-ring constriction and septum synthesis combine to drive cytokinesis. As FtsW, FtsI, and FtsN are transmembrane proteins with cytoplasmic and periplasmic domains, they could coordinate the Z-ring and nascent septum, with SidA and DidA disrupting this coordination. Assembly or activity of the FtsW/FtsI/FtsN subcomplex could somehow trigger FtsZ constriction, and the inhibitors SidA and DidA may block this step of division. Finally, it is possible that Z-ring constriction and septum synthesis combine to drive cytokinesis. As FtsW, FtsI, and FtsN are transmembrane proteins with cytoplasmic and periplasmic domains, they could coordinate the Z-ring and nascent septum, with SidA and DidA disrupting this coordination. Distinguishing between these various models for cytokinesis and elucidating the precise mechanisms of action for SidA and DidA will ultimately require more detailed studies of the FtsW/I/N subcomplex; the mutants identified here, such as FtsW(A246T), may prove particularly useful in these efforts.

Final Perspectives

Our results (i) reveal an SOS-independent mechanism for inhibiting cell division in Caulobacter and (ii) highlight the FtsW/FtsI/FtsN subcomplex as an important regulatory node in the control of cell division. Following certain types of DNA damage, DidA and SidA appear to function together to prevent inappropriate cell divisions (Figure 8). Such redundancy may afford cells with a fail-safe survival mechanism. In addition, SidA and DidA are differentially induced following different types of DNA damage, providing independent routes to the inhibition of cell division under different conditions. Also, we note that although cells lacking both sidA and didA divide prematurely during DNA damage, many still filament to some degree, suggesting that yet other mechanisms of division inhibition exist in Caulobacter. Finally, we note that DidA is the latest in a growing class of small, stress-induced membrane proteins that play critical regulatory roles [50,51]. These proteins are often missed or incorrectly annotated in genome sequences, but many, like SidA and DidA, clearly play critical roles in regulating cellular processes, including cell division.

Materials and Methods

Strains, Plasmids, and Growth Conditions

Strains and plasmids used in this study are listed in Table S1 with construction details and growth conditions provided in Text S1.

Synchronization

Synchronous Caulobacter populations were obtained by centrifugation over a Percoll density gradient as previously described [52]. Following synchronization of the ftsW(A246T) strain, we noticed that 21% of cells (two biological replicates) were unable to form microcolonies on plain PYE agarose pads compared to 2% for wild-type cells. Because of this sensitivity to the synchronization procedure, ftsW(A246T) cells and other suppressors were imaged by time-lapse microscopy following growth in mixed cultures.

Figure 8. Two independent pathways regulate cell division in Caulobacter following DNA damage. (A–B) Two cell division inhibitors are induced following DNA damage in Caulobacter. sidA is induced by cleavage of the SOS repressor LexA while didA is induced by DriD. SidA and DidA are small transmembrane proteins that can block cell division by preventing the divisome subcomplex FtsW/I/N from assuming an active state, designated FtsW/I/N*. FtsW/I/N* could promote division by enhancing peptidoglycan synthesis and remodeling, by triggering FtsZ constriction, or by coordinating these activities.

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overnight in PYE and plated on PYE agar supplemented with overproduction plasmid and plated on PYE agar in absence of vanillate and samples were taken for immunoblots, plasmid fractionation was performed as described [13].

Immunoblots and Biochemical Fractionations
Samples for immunoblots were normalized in sample buffer to 0.5 OD600/50 μl, resolved on 12% sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Membranes were probed with polyclonal rabbit α-CtrA, α-DivI, α-LacZ (Rockland Scientific), and α-GFP (Invitrogen) at a 1:5,000 dilution and monoclonal mouse α-FLAG (Sigma) at a 1:3,000 dilution. Secondary HRP-conjugated α-rabbit (Pierce) or α-mouse (Pierce) were used at a 1:5,000 dilution. Blots were visualized by chemiluminescence; raw black-and-white images were inverted for display. Biochemical fractionation was performed as described [13].

Microscopy
All phase contrast images were acquired on a Zeiss Observer Z1 microscope with a 100 x 1.4 oil immersion objective and an LED-based CoLiBi illumination system. For additional information on image analysis and time-lapse microscopy, see Text S1.

Bacterial Two-Hybrid Analysis
Two-hybrid complementation assays were performed essentially as described [16]. BTH101 cells harboring plasmids with the T25 and T18 fusion constructs were grown to single colonies on LB agar plates and restruck or spotted on MacConkey agar plates supplemented with maltose for imaging.

ftsN Mutagenesis Screen
The ftsN mutagenesis PCR reaction contained 21 μl 3M Betaine, 1 μl DMSO, 5 μl 10× Taq buffer (Invitrogen), 1.5 μl 50 mM MgCl2, 4 μl dNTPs, 0.2 μl primers, 50 ng genomic DNA, 2 μl mutagenesis buffer (100 mM dCTP, 100 mM dTTP, 50 mM MgCl2, 500 mM MnCl2), 0.3 μl Taq polymerase (Invitrogen), and water to 50 μl. The PCR reaction was incubated at 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 58°C for 1 minute, and 72°C for 3 minutes with a final extension of 72°C for 10 minutes. The mutant ftsN library was then cloned into a medium-copy plasmid downstream of the xylose-inducible promoter.

An ftsN depletion strain harboring a low-copy plasmid expressing 3 × M2-didA from Pbac was transformed with a medium-copy plasmid expressing the mutant ftsN library from Pstd and grown on plates containing oxytetracycline, kanamycin, and 75 or 100 μM IPTG. The medium-copy kanamycin-resistant plasmids from suppressor colonies were isolated and restested in a clean ftsN depletion background for their ability to suppress 3 × M2-didA overexpression from the IPTG-inducible low-copy plasmid. ftsN mutations in suppressor plasmids were identified by Sanger sequencing.

Identification of DidA Overproduction Supressors
Wild-type cells were transformed with a Pbac-3 × M2-didA overproduction plasmid and plated on PYE agar in absence of vanillate to allow colony formation. Single colonies were grown overnight in PYE and plated on PYE agar supplemented with vanillate at roughly 2 × 10^6 colony forming units per 10 cm plate. Rare colonies were grown overnight in PYE supplemented with vanillate and samples were taken for immunoblots, plasmid preparations, and archiving. To isolate chromosomal suppressor mutations and eliminate mutations arising in the 3 × M2-didA overproduction plasmid, we screened for colonies that met two criteria. (1) We used immunoblotting to check that 3 × M2-DidA production in each suppressor strain was similar to that seen in wild-type cells transformed with the same plasmid and grown in vanillate for 1.5 h. (2) Plasmids from the suppressor strains were transformed into wild-type cells and plated on PYE agar supplemented with or without vanillate. The presence of thousands of colonies on plain plates and few colonies on vanillate indicated a functional plasmid. The mutation in the ftsW (A246T) suppressor strain was identified by whole genome resequencing.

Screen for Activators of didA Expression
Cells expressing lacZ from ΦΔidA at the hfsB locus in a ΔdidA background were mutagenized with the EZ-Tn5 transposome (Epicentre) and grown on plates containing kanamycin and 20 μg/ml X-gal. Colonies appearing white were isolated and tested for low or undetectable levels of full-length LacZ by western blot with α-LacZ antibodies. Transposon-insertion mutations were identified as described (Epicentre, TSM08KR protocol) by rescue cloning with pnr-116 electroporant E. coli cells (Epicentre).

ChiP and Quantitative PCR Analysis
ChiP was performed as detailed in Text S1. Quantitative PCR was performed with the dye SYBR Green (Roche) on a Lightcycler 480 system (Roche). Each reaction contained 5 μl SYBR Green Master, 1 μl DNA (diluted 1:500 for pre-ChiP input DNA, and 1:20 for post-ChiP output DNA), 0.5 μl primer mix at 10 μM, and 5.5 μl nuclease-free water. Primers amplifying a product within the ruvA coding sequence were used as a control. Cycle threshold values were calculated using the Lightcycler 480 software and converted to DNA concentrations based on a standard curve generated from 2-fold dilutions of Caulobacter genomic DNA. Fold enrichment values were calculated as [Pstd−output]/[Pstd−input]/[ΦΔidA−output]/[ΦΔidA−input]. Error bars in Figure 7F were generated from technical triplicates, and the experiment shown is representative of biological duplicates.

Supporting Information
Figure S1 Cellular filamentation of sidA and recA mutants. Wild-type, ΔsidA, and ΔrecA cells were grown to mid-exponential in rich media and treated with 1 μg/ml MMC or left untreated. After 3 hours, cells were imaged by phase microscopy. Bar, 2 μm. (TIF)

Figure S2 Annotated gene expression profiles. (A) Transcriptional profiles for the 50 most upregulated genes during DNA damage in wild-type cells (see Figure 1A) are shown with their corresponding CC numbers and NA1000 annotation. The “LexA” column shows genes whose upstream region contains a sequence match to 7 of the 8 bases in the Caulobacter LexA consensus binding site (GTTCN7GTTC) [15]. Genes whose log-fold changes post-damage in ΔrecA cells are below 50% of those in wild-type cells are marked as “RecA-dependent.” All other genes are marked as “RecA-independent.” (B) The positions of microarray probes within CC3114 and CCNA03212 are shown below the genes as horizontal bars. The four right-most probes are used to calculate expression values for CCNA03212 (didA). (C) The transcriptional profiles for each probe in (B) are shown. (TIF)

Figure S3 DNA damage induction of DidA. (A) Cells expressing 3 × M2-didA from the native, chromosomal Pstd
promoter were exposed to 1 or 3 μg/ml MMC or left untreated. Wild-type cells harboring a low- (pCT133) or medium- (pCT155) copy plasmid expressing $3 \times M2$-DidA from $P_{\text{van}}$ were treated with or without vanillate. After 3 hours, cells were imaged by phase microscopy. Bar, 2 μm. (B) Samples from the experiments in (A) were taken at the times indicated and analyzed by Western blot using an α-FLAG/M2 antibody.

**Figure S4** DidA interacts with FtsN. Bacterial two-hybrid analysis of interactions between T25-DidA (A) or T18-DidA (B) and cell division proteins fused to T18 or T25, respectively. Each pair was plated on LB, and colonies were restruck on MacConkey plates containing maltose.

**Figure S5** SidA interacts with FtsW. (A) Cells expressing wild-type $fbsW$ or $fbsW(A246T)$ and overproducing M2-YFP-DidA for 2.5 hours were imaged by phase and epifluorescence microscopy. (B) Bacterial two-hybrid analysis of interactions between T18-M2-SidA and FtsW mutants fused to T25 as indicated. Colonies were grown to exponential phase in LB and 5 μl aliquots plated on MacConkey containing maltose. (TIF)

**Figure S6** Suppressor mutant growth properties. (A) Growth curves for the strains from Figure 5D grown in rich media. (B) Wild-type and $fbsW(A246T)$ cells were grown to mid-exponential phase and imaged by phase microscopy. Cell lengths were quantified from 491 wild-type and 610 $fbsW(A246T)$ cells using MicrobeTracker and summarized as a histogram with the maximum frequency for each strain normalized to 1. (C) Growth curves for wild-type, $fbsW(A246T)$ and $fbsW^{\ast\ast\ast}$ cells grown in rich media. (D) Mixed populations of wild-type and $fbsW(A246T)$ cells ($n\sim200$) were imaged by timelapse microscopy on PYE agarose pads. The times to first mid-cell division are shown. For raw data, see Data S3. (TIF)

**Figure S7** $fbsW(A246T)$ cells divide prematurely during MMC exposure. Mixed populations of wild-type and $fbsW(A246T)$ cells ($n\sim100$) were imaged by time-lapse microscopy on PYE agarose pads containing 0.35 μg/ml MMC. The time to first mid-cell division and the percentage of cells that stopped growing following division are shown. Asterisks represent a statistically significant ($p<0.01$) difference relative to the wild type. Error bars represent standard error of the mean. For raw data, see Data S3. (TIF)

**Figure S8** Induction at $P_{\text{sidA}}$ and $P_{\text{didA}}$. Wild-type cells harboring low-copy plasmids transcribing egfp from either $P_{\text{sidA}}$ or $P_{\text{didA}}$ were exposed to MMC (0.35 or 1.75 μg/ml) or cephalixin (5 or 35 μg/ml) for 1.5 or 3 hours. Samples were analyzed by Western blot with an α-EGFP antibody.

**Figure S9** Suppressors treated with cephalixin exhibit cell wall defects. The strains from Figure 6D, grown to mid-exponential phase in rich media and treated with MMC or cephalixin for 6 hours and PI at 5 μM 1.5 hours before imaging. Cells were imaged by phase and fluorescence microscopy; representative populations are shown with PI+ cells false-colored red. Bar, 2 μm. (TIF)

**Figure S10** Induction of didA during stress conditions. Cells expressing didA-3 X M2 from the native, chromosomal didA promoter were treated with 3 μg/ml MMC, 1 and 3 mg/ml hydroxyurea (HU), 36 μg/ml cephalixin (ceph), and 10 and 100 μg/ml novobiocin (nov) for 1 hour each, ultraviolet light using a Stratalinker at energy setting 100 and 300 (UV), grown overnight in minimal medium (M2G), starved of glucose in minimal medium (− glu) for 30, 60, and 90 minutes, or treated with 5% and 10% ethanol (EtOH), 50 and 200 mM NaCl, 10 and 100 mM hydrogen peroxide (H2O2), 5 μg/ml kanamycin (kan), 1 μg/ml oxytetracycline (Tet), or 2 μg/ml chloramphenicol (chlor) for 45 minutes each. Samples were analyzed by Western blot using an α-FLAG/M2 antibody.

**Table S1** Strains, plasmids, and primers. (XLSX)

**Data S1** Microarray data for (A) wt and ΔrecA cells treated with 1 μg/ml MMC for 30 minutes and (B) wt cells harboring pCT155: $P_{\text{van}}$-didA treated with or without vanillate for 45 minutes. (XLSX)

**Data S2** Summary of growth and division defects following MMC treatment. (XLSX)

**Data S3** Raw data from Figures 6, 7, S6, and S7. (XLSX)

**Text S1** Extended materials and methods. (DOCX)

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**Author Contributions**

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: JWM MTL. Performed the experiments: JWM MTL. Analyzed the data: JWM MTL. Contributed reagents/materials/analysis tools: JWM MTL. Wrote the paper: JWM MTL.


