Cyclic di-GMP acts as a cell cycle oscillator to drive chromosome replication

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1   **Summary:**

2   Fundamental to all living organisms is the capacity to coordinate cell division and cell

3   differentiation to generate appropriate numbers of specialized cells. While eukaryotes

4   use cyclins and cyclin-dependent kinases to balance division with cell fate decisions,

5   equivalent regulatory systems have not been described in bacteria. Moreover, the

6   mechanisms used by bacteria to tune division with developmental programs are poor-

7   ly understood. Here we demonstrate that *Caulobacter crescentus*, a bacterium with an

8   asymmetric division cycle, uses oscillating levels of the second messenger c-di-GMP

9   to drive its cell cycle. We demonstrate that c-di-GMP directly binds to the essential

10 cell cycle kinase CckA to inhibit kinase and stimulate phosphatase activity. An up-

11 shift of c-di-GMP during the G1-S transition switches CckA from the kinase into the

12 phosphatase mode, thereby licensing replication initiation and cell cycle progression.

13 Finally, we show that during division, c-di-GMP imposes spatial control on CckA to

14 install replication asymmetry of future daughter cells. These studies expose c-di-GMP

15 as a cyclin-like molecule in bacteria that coordinates chromosome replication with

16 cell morphogenesis in *Caulobacter*. The observation that c-di-GMP mediated control

17 is conserved in the plant pathogen *Agrobacterium tumefaciens* unfolds a general

18 mechanism through which this global regulator of bacterial virulence and persistence

19 coordinates behaviour and cell proliferation.

20

1   To enable tissue homeostasis, metazoans tightly regulate the balance between cell

2   proliferation and differentiation[1]. Central players in cell proliferation, development

3   and cell fate decisions are cyclin-dependent kinases (CDKs)[2,3]. To drive cell cycle

4   progression, CDKs associate with oscillating, stage-specific regulatory subunits called

5   cyclins[4]. While in higher organisms cells generally undergo terminal differentiation,

6   bacteria often rely on rapid growth to exploit available nutrients and thus need to dy-

7   namically tune behavioural programs with cell proliferation. How exactly bacteria

8   couple behavioural processes with cell cycle progression remains unclear.

9   A prime model to study the coupling of cell growth and behaviour in bacteria is the

10   aquatic organism *Caulobacter crescentus*, which strictly separates cell motility from

11   cell proliferation. *C. crescentus* divides asymmetrically to generate two specialized

12   progeny, a sessile and replication competent stalked and a motile and replication inert

13   swarmer cell. The swarmer cell (G1-phase) re-enters the replication cycle during dif-

14   ferentiation into a stalked cell (S-phase) (Figure 1a). To control the motile-sessile

15   transition, *C. crescentus* makes use of c-di-GMP, a second messenger controlling a

16   wide range of behavioural processes in bacteria, including virulence, motility and bio-

17   film formation[5]. C-di-GMP levels are low in swarmer cells, increase during differen-

18   tiation to peak in stalked cells and later reach intermediate levels in the predivisional

19   cell[6]. One of the main drivers of c-di-GMP fluctuations is the diguanylate cyclase

20   PleD, which is active in stalked but turned off in swarmer cells[7] (Figure 1a). While a

21   *pleD* mutant has reduced levels of c-di-GMP, a strain lacking all diguanylate cyclases

22   (cdG0) is devoid of c-di-GMP[6]. The complete loss of motility and surface attachment

23   in the cdG0 strain illustrates the importance of c-di-GMP oscillation for *Caulobacter*

24   cell fate determination[6]. In contrast, the role of c-di-GMP in cell cycle progression is

25   unclear.

1   Our studies originated from a genetic screen for synthetic lethal mutants in the cdG0

2   background. This strain, although viable, shows pronounced morphology and cell cy-

3   cle defects[6]. We thus reasoned that c-di-GMP controls cell cycle progression together

4   with a parallel pathway with partial functional redundancy. The screen revealed a

5   strain with a transposon (Tn) insertion in the promoter region of the gene encoding the

6   single-domain response regulator DivK (P*divK*::Tn) (Figure S1a). Crossing back the

7   Tn into the cdG0 mutant produced a strain with severe cell cycle defects (Figure 1b,

8   S1b). This, and the observation that the DNA content per cell mass unit was severely

9   reduced (Figure 1c), indicated that cells are severely compromised for replication ini-

10   tiation. In contrast, growth, division and replication were not affected when the

11   P*divK*::Tn was crossed into a cdG$^+$ strain (Figure 1b,c). DivK levels were reduced

12   about 10-fold in the cdG0 P*divK*::Tn strain (Figure S1c), suggesting that DivK may be

13   limiting for growth. This was confirmed by replacing the *divK* promoter upstream of

14   the *divK* gene with the xylose-dependent promoter P*xyl*. In the absence of the inducer

15   or in the presence of glucose, which further represses Pxyl activity, DivK levels were

16   strongly reduced compared to wild type (Figure S1d), resulting in severely reduced

17   growth and replication in the cdG0 strain, but not in a cdG$^+$ background (Figure 1b,

18   S1e). Together, this indicated that c-di-GMP and DivK convergently regulate cell cy-

19   cle progression.

20   DivK was recently shown to down-regulate the central cell cycle kinase CckA

21   through a direct interaction with DivL, an unorthodox kinase that controls CckA via

22   protein protein interaction[8,9]. CckA initiates a phosphorelay controlling the activity of

23   the response regulator CtrA[10,11] (Figure 1a). CtrA is phosphorylated and active in

24   swarmer cells (G1) where it binds to the origin of replication (Cori) to inhibit replica-

25   tion initiation[12]. During differentiation into stalked cells CtrA is inactivated to license

1 replication initiation[13]. CckA is bifunctional and can act both as kinase and as phos-

2 phatase to control CtrA via the phosphotransfer protein ChpT[14]. Accordingly, switch-

3 ing CckA from kinase to phosphatase activity during G1-S would rapidly reverse the

4 phosphate flux to inactivate CtrA and authorize replication initiation. Hence, we rea-

5 soned that DivK and c-di-GMP could cooperate to inactivate CtrA. Because c-di-

6 GMP controls CtrA degradation during G1-S transition through the effector protein

7 PopA[15] (Figure 1a), the G1 arrest of the cdG0 *PdivK*::Tn strain could conceivably re-

8 sult from simultaneous over-activation and stabilization of CtrA. However, a mutant

9 combining the *PdivK*::Tn allele with a *popA* deletion stabilizing CtrA was not affected

10 in growth or DNA replication. In contrast, a *PdivK*::Tn Δ*popA* strain that also lacked

11 PleD, produced a strong G1 arrest (Figure 1c, S1f). From this we concluded that c-di-

12 GMP regulates both stability and phosphorylation levels of CtrA during the cell cycle

13 (Figure 1a).

14 To analyse how c-di-GMP regulates CtrA activity, individual components of the

15 CckA-CtrA phosphorelay were purified and examined *in vitro*. In the absence of c-di-

16 GMP CckA autophosphorylation and phosphotransfer via ChpT to CtrA were readily

17 observed. Strikingly, the addition of c-di-GMP completely abolished phosphorylation

18 of all three components (Figure S2a). When CckA auto-phosphorylation was first car-

19 ried out in the absence of c-di-GMP followed by the addition of c-di-GMP to the reac-

20 tion mixture, rapid dephosphorylation of CckA was observed, arguing that c-di-GMP

21 is a potent stimulator of CckA phosphatase activity (Figure 2a, S2b). Stimulation of

22 the CckA phosphatase was specific to c-di-GMP with GMP, GTP or cGMP having no

23 observable effect (Figure 2a). Experiments with all three components of the phos-

24 phorelay demonstrated that c-di-GMP effectively reverses the phosphate flux of the

25 phosphorelay leading to the inactivation of CtrA (Figure 2b). To test if c-di-GMP also

1   regulates CckA kinase activity we compared phosphorylation of wild-type CckA with

2   CckA(V366P), a mutant lacking phosphatase activity *in vitro* (Figure S2c)[14]. When c-

3   di-GMP was added together with [^{32}P]ATP at reaction start, CckA(V366P) phosphor-

4   ylation was strongly reduced as compared to a control lacking c-di-GMP (Figure

5   S2c), indicating that c-di-GMP inhibits CckA kinase activity.

6   These experiments demonstrated that c-di-GMP is a potent trigger to switch CckA

7   from its default kinase into the phosphatase state. Consistent with this, purified CckA

8   specifically binds radiolabeled c-di-GMP (Figure 2c, S2d). Further studies exposed

9   the catalytic ATP-binding domain (CA) as minimal binding region for c-di-GMP

10   (Figure S2e-g). To identify amino acid residues of the CA domain that are specifically

11   involved in c-di-GMP binding, we concentrated on a candidate mutation that was re-

12   cently isolated in the CckA homolog of the plant pathogen *Agrobacterium tumefa-*

13   *ciens* (CckA$_{At}$). In this organism a spontaneous Y674D substitution in the CA domain

14   of CckA$_{At}$ was isolated as a motile suppressor of a mutant lacking PleC[16]. We hypoth-

15   esized that CckA$_{At}$ is also regulated by c-di-GMP and that in a *pleC* mutant with ele-

16   vated levels of c-di-GMP (Figure 1a) the Y674D mutation restores its ki-

17   nase/phosphatase balance by interfering with c-di-GMP binding. As shown in Figure

18   S2h, autophosphorylation of purified wild-type CckA$_{At}$ was specifically reversed

19   when c-di-GMP was added, while the CckA$_{At}$(Y674D) mutant failed to respond to c-

20   di-GMP. Moreover, c-di-GMP binding to CckA$_{At}$(Y674D) was strongly reduced as

21   compared to the wild-type form of the protein (Figure S2h). The equivalent substitu-

22   tion in *Caulobacter* CckA (Y514D) also resulted in strongly diminished c-di-GMP

23   binding (Figure 2c). Importantly, the CckA(Y514D) mutant showed normal kinase

24   activity but failed to dephosphorylate upon addition of c-di-GMP (Figure 2d). This

25   was not due to a general lack of phosphatase activity, as the CckA(Y514D) mutant

1   showed unaltered basal level phosphatase activity upon ATP depletion (Figure S2i).

2   Together this demonstrated that CckA(Y514D) is compromised for c-di-GMP binding

3   and, as a consequence, cannot switch to the phosphatase mode upon addition of c-di-

4   GMP, resulting in constitutive CckA kinase activity *in vitro*. To define the c-di-GMP

5   binding pocket on the surface of the CA domain we used a combination of structural

6   modelling, biochemical analysis and NMR spectroscopy (Figures 2e, S2j-l). This ap-

7   proach identified a set of six amino acids, F474, F493, Y514, W523, R537, and F539,

8   which show significant NMR chemical shift perturbations upon c-di-GMP titration

9   experiments and are strictly required for c-di-GMP binding and phosphatase but not

10   kinase activity (Figure 2e, S2i,l). All of these residues locate in close proximity of

11   Y514 in a homology model of CckA (Figure 2e). Interestingly, six of these amino ac-

12   id residues feature aromatic side chains and are well conserved in CckA homologs

13   (Figure S2m). This argues that c-di-GMP is coordinated by the CA domain of CckA

14   via hydrophobic interactions, akin to the binding mode described for the human

15   STING receptor[17].

16   Next we set out to test if c-di-GMP executes its important cell cycle role primarily by

17   interfering with the CckA kinase/phosphatase balance *in vivo*. We reasoned that a

18   combination of P*divK*::Tn and *cckA*(*Y514D*) should cause a similar G1 arrest as ob-

19   served for a strain lacking c-di-GMP altogether. Moreover, this combination should

20   lead to a cell cycle arrest irrespective of the presence of c-di-GMP (Figure S3a). In-

21   deed, cells carrying P*divK*::Tn and *cckA*(*Y514D*) showed severe growth defects (Fig-

22   ure 3a), increased binding of CtrA to the Cori region (Figure S3b), and a strong G1

23   arrest (Figure 3b, S3c). While this phenotype was independent of PleD, viability of

24   the CckA phosphatase mutant (V366P) strictly depended on c-di-GMP (Figure 3a,b,

25   S3c). This indicated that down-regulation of CckA kinase activity by c-di-GMP is

1 sufficient to balance the kinase/phosphatase activities of the V366P mutant. To cor-

2 roborate these findings we tested the same *cckA* alleles in strains expressing *divK*

3 from the xylose-dependent promoter P*xyl*. When P*xyl*::*divK* cells were grown in the

4 absence of xylose, DivK dropped below 10% of wild type and, as a consequence,

5 cells developed a mild G1 arrest (Figure S3d). This effect was aggravated in strains

6 expressing *cckA*(*Y514D*) resulting in a strong reduction of the DNA/cell mass ratio

7 (Figure S3d), severely reduced growth (Figure S3e), increased CckA~P phosphoryla-

8 tion levels (Figure S3f) and an overall reduction of the number of chromosomal ori-

9 gins per cell mass (Figure S3g).

10 Taken together, these experiments lead us to propose a model where two convergent

11 regulatory inputs, DivK and c-di-GMP, control the CckA kinase/phosphatase switch

12 to authorize G1-S transition through the inactivation of the replication initiation inhib-

13 itor CtrA (Figure 1a). Intriguingly, cell type specific activity of both PleD and DivK is

14 regulated by DivJ and PleC, two histidine kinase/phosphatase antagonists, which lo-

15 calize to opposite poles of the predivisional cell and during division asymmetrically

16 partition into the daughter cells to determine their respective programs (Figure 1a)[18].

17 Thus, the two regulators show similar activation profiles during the cell cycle[7,19],

18 thereby imposing tight coordination between the DivK branch and the c-di-GMP

19 branch of the CckA switch. This connection is further strengthened by the role of

20 DivK as allosteric activator of the DivJ kinase, a positive feedback mechanism

21 through which both DivK and PleD activity can be rapidly upregulated during G1-S

22 transition[19]. Hence, DivK and c-di-GMP act as molecular connectors between two

23 hierarchical phosphorylation modules, explaining how the cellular dynamics of PleC

24 and DivJ translate into differential activities of the central cell cycle kinase CckA

25 (Figure 4). Because the parallel morphogenetic program critically depends on PleD

1   activation and the concomitant rise in c-di-GMP concentration[6], c-di-GMP induced

2   inactivation of CtrA directly couples development to cell cycle progression. This is

3   reminiscent of redundant pathways regulating cell cycle progression in higher eukary-

4   otes, where a multitude of signals converge to control the activity of CDKs[20,21].

5   In addition to its role in G1-S transition, CckA facilitates cell polarity during division.

6   CckA localizes to both poles of dividing *Caulobacter* cells but adopts differential ki-

7   nase/phosphatase activities at opposite poles[14,22] (Figure 1a). The resulting cellular

8   gradient of CtrA~P was proposed to establish asymmetric replication activities, which

9   propagate to future daughter cells[23]. To test if c-di-GMP contributes to replication

10   asymmetry during division we made use of fluorescent repressor-operator systems

11   (FROS) to spatially resolve replication initiation events (Figure S4a,b)[24]. While in a

12   majority of wild-type cells chromosome replication originated at the old stalked pole,

13   cells expressing *cckA(V366P)* or *cckA(Y514D)* lost replication asymmetry almost en-

14   tirely (Figure S4c). Cells lacking PleD also partially lost their replication preference

15   for the stalked pole. Because active PleD, PleD~P, specifically localizes to the stalked

16   pole[7,25], we analysed replicative asymmetry in a cdG0 strain expressing a heterolo-

17   gous diguanylate cyclase, DgcZ, from *E. coli*, which is uniformly distributed in the

18   cell[6,26]. Although expression of *dgcZ* restored all developmental defects in this strain[6],

19   it failed to establish the characteristic spatial replication bias (Figure S4c). From this

20   we conclude that the spatial organization of c-di-GMP metabolism contributes to cell

21   polarity by differentially regulating CckA at opposite cell poles. For example, a local

22   environment with high levels of c-di-GMP might impose CckA phosphatase activity

23   at the stalked pole. Alternatively, a local trough of c-di-GMP may exist at the swarm-

24   er pole with the rest of the cell body containing high levels of c-di-GMP. To distin-

25   guish between these possibilities, we made use of a CckA variant that is unable to lo-

1   calize to cell poles because it lacks its membrane anchor (*cckAΔTM*). Expression of

2   this mutant causes massive over-replication and cell filamentation, arguing that delo-

3   calized CckA functions primarily as a phosphatase for CtrA[14]. In agreement with this,

4   expression of *cckAΔTM(V366P),* lacking phosphatase activity, did not show any ad-

5   verse effects (Figure S4d). Strikingly, expression of *cckAΔTM(Y514D)* in a cdG$^+$

6   strain (Figure S4d) or expression of *cckAΔTM* in a strain lacking c-di-GMP (Figure

7   S4e) led to a strong G1 arrest, a hallmark of the CckA kinase mode. This indicated

8   that the cellular pool of c-di-GMP strictly imposes phosphatase activity on delocal-

9   ized CckA molecules.

10   Based on these results we propose that the bulk volume of dividing *C. crescentus* cells

11   experiences high levels of c-di-GMP and that CckA adopts strong kinase activity at

12   the swarmer pole as a consequence of a microenvironment with low levels of c-di-

13   GMP. This view is consistent with the idea that sequestration of CckA to the swarmer

14   pole creates a microenvironment within the cell where CckA can avoid down-

15   regulation by its other inhibitor, DivK~P[9,23]. We propose that CckA sequestration to

16   this subcellular site also shields the protein from the cellular pool of c-di-GMP. Ulti-

17   mately, it is the PleC phosphatase that reduces PleD~P and DivK~P levels at this sub-

18   cellular site and, possibly together with one or several swarmer pole specific phos-

19   phodiesterases, imposes this spatial regime (Figure 1a, 4). The input from c-di-GMP

20   might also explain how the entire cellular pool of CckA can be tightly regulated.

21   Throughout the cell cycle, CckA localization is often patchy and dynamic without be-

22   ing strictly limited to polar regions[22]. Since the degree of co-localization of DivK and

23   CckA is unclear, c-di-GMP could effectively maintain CckA in the phosphatase state

24   in all cell types or subcellular regions harbouring high levels the second messenger.

1   C-di-GMP is only one of several novel nucleotide-based second messengers that were

2   recently discovered in bacteria[27]. Their global impact on cell physiology raised the

3   question how these signalling compounds mediate specific cellular responses and how

4   they integrate with other general signalling systems, in particular with two-component

5   phosphorylation networks[28]. Our finding that c-di-GMP acts as a cyclin-like molecule

6   in *C. crescentus* to control the activity of the cell cycle kinase CckA, establishes the

7   first direct connection between the two most widespread regulatory networks of bac-

8   terial cells. The CckA-ChpT-CtrA pathway is conserved among most known mem-

9   bers of the alpha-proteobacteria, including important pathogens like *Bartonella* or

10   *Brucella*[29]. This opens up the exciting possibility that c-di-GMP-imposed control of

11   sensor histidine kinases might represent a general and widespread regulatory mecha-

12   nism in bacteria. Considering that c-di-GMP plays a major role in regulating virulence

13   and persistence, this provides important new entry points into better understanding the

14   behaviour and propagation of bacterial pathogens.

15

8   **Author contributions**

9   C.L., S.O., S.A., S.S. and U.J. initiated the project. All authors designed experiments.

10 S.O. carried out genetic experiments. C.L. and S.S. carried out biochemical experi-

11 ments. C.L. and S.O. carried out microscopy experiments. R.B. and S.H. carried out

12 NMR experiments. B.N.D. and T.S. contributed to structural analysis. U.J., S.O., and

13 C.L. wrote the manuscript.

14

1 **Figure legends:**

2 **Figure 1: C-di-GMP regulates cell cycle progression via the CckA-CtrA phos-**
3 **phorelay.**

4 **(a)** Left: Localization of CckA and factors regulating CckA activity throughout the *C.*

5 *crescentus* cell cycle. CckA kinase (red) and phosphatase (blue) activities are indicat-

6 ed. High and low levels of c-di-GMP are shown as grey or white areas, respectively.

7 PDE=phosphodiesterase. Right: Regulatory modules inactivating CtrA to control

8 *Caulobacter* S-phase entry. **(b)** Growth (left) and cell morphology (right) of strains

9 indicated. 5-fold serial dilutions are shown. P*xyl*::*divK* strains were grown on PYE

10 (none) or PYE glucose (Gluc) plates. **(c)** Effect of P*divK*::Tn on DNA replication in

11 different genetic backgrounds. DNA content and cell mass were determined by flow

12 cytometry. Averages and standard deviations for DNA content/cell mass were ob-

13 tained from 4 biological replicates.

14

15 **Figure 2: C-di-GMP binds to the catalytic domain to induce CckA phosphatase**
16 **activity.**

17 **(a)** C-di-GMP specifically stimulates CckA dephosphorylation. CckA phosphoryla-

18 tion reactions were started by adding [^{32}P]ATP (0 min) and supplemented with c-di-

19 GMP (75 µM) at the time indicated (arrow). The inlet shows CckA phosphorylation

20 reactions supplemented with c-di-GMP and other nucleotides (75 µM). **(b)** C-di-GMP

21 reverses the phosphate flux of the CckA-ChpT-CtrA phosphorelay. Reactions were

22 run for 30 min and c-di-GMP was added together with [^{32}P]ATP at time 0 min (t0) or

23 15 min after reaction start (t15). **(c)** C-di-GMP binding affinity of CckA. Binding of

24 wild-type CckA (WT) and CckA(Y514D) was determined by UV-crosslinking at in-

25 creasing concentrations of [^{33}P]c-di-GMP (inlet) and quantified as shown in the graph.

Averages and standard deviations were obtained from three replicates. **(d)** C-di-GMP

fails to stimulate phosphatase activity of the CckA(Y514D) mutant. Phosphorylation

reactions with wild-type CckA (WT) and Y514D mutant protein were analysed with-

out (none) or with c-di-GMP added at time point 0 (t0) or after 15 min (t15). **(e)** Ho-

mology model of the CA domain of CckA based on a crystal structure of DivL (pdb

4q20). Residues that show large ($\Delta\delta$(HN) > 2 s.d.) and intermediate (2 s.d. >

$\Delta\delta$(HN) > 1 s.d.) amide chemical shift perturbations upon addition of c-di-GMP

are shown in purple and pink, respectively. Side chains of residues that contrib-

ute to c-di-GMP binding and c-di-GMP mediated phosphatase activity are shown

in stick representation and coloured in red. A single molecule of ATP (yellow)

was modelled into its putative binding site based on homology to CpxA

(pdb4bix). D479, which is involved in ATP binding, is shown green. For more in-

formation, see legend to Figures S2k,l.

Figure 3: C-di-GMP controls CckA activity to initiate chromosome replication.

(a) The *cckA(Y514D)* allele shows synthetic lethality with P*divK*::Tn. 5-fold serial

dilutions of strains containing combinations of *cckA*, P*divK*, and *pleD* alleles were

incubated on PYE plates for 2 days. **(b)** Combining the *cckA(Y514D)* and P*divK*::Tn

alleles leads to a G1 arrest. Exponential cultures of mutants containing combinations

of *cckA,* P*divK*, and *pleD* alleles were analysed by flow cytometry. Values of DNA

content per cell mass are shown relative to *C. crescentus* wild type and were obtained

as described in the legend for Figure 1c. Averages and standard deviations were ob-

tained from three replicates.

1 **Figure 4: Model of the regulatory circuitry controlling cell cycle progression in**
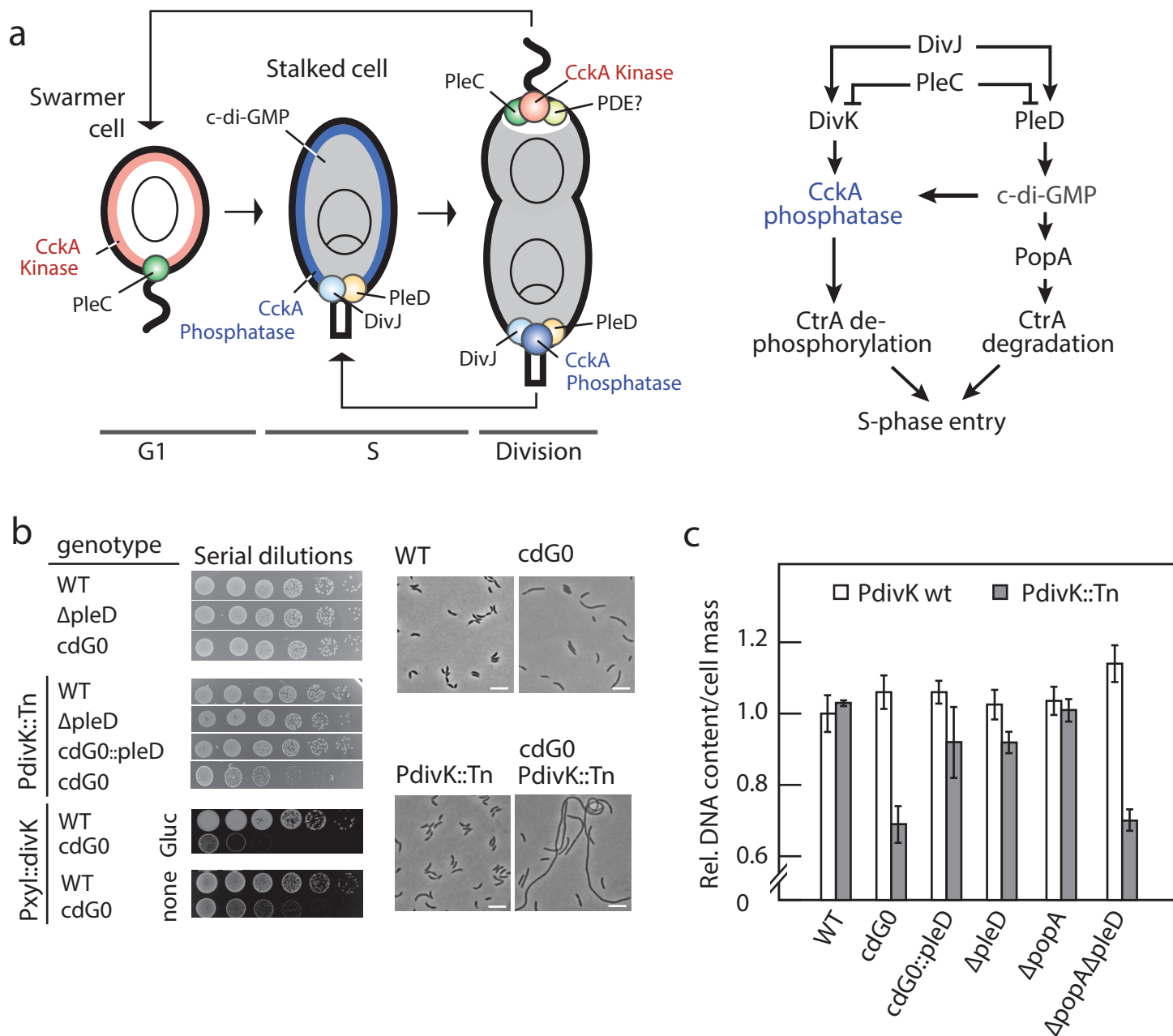
2 ***C. crescentus*.**

3 Two intercalated phosphorylation modules control replication initiation through the

4 activity of the replication initiation inhibitor CtrA. When the PleC phosphatase is pre-

5 sent at the swarmer pole, PleD and DivK are dephosphorylated. In this situation,

6 phosphorylation modules 1 and 2 are uncoupled and CckA adopts DivL-imposed ki-

7 nase mode to activate CtrA and block replication initiation. When the DivJ sensor ki-

8 nase is present at the stalked pole, phosphorylation module 1 imposes control on

9 module 2. PleD and DivK are phosphorylated, thereby switching CckA into the phos-

10 phatase mode and inactivating CtrA. In parallel, c-di-GMP facilitates CtrA degrada-
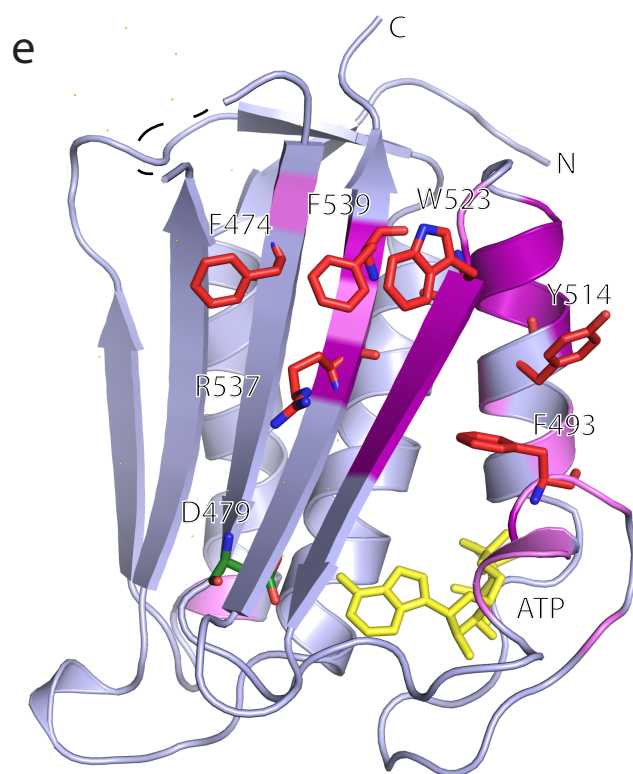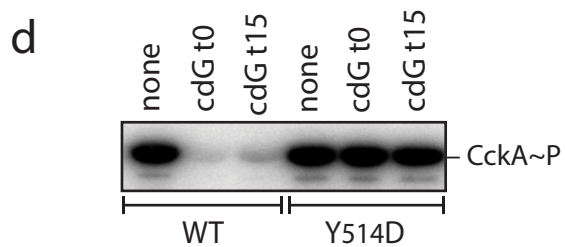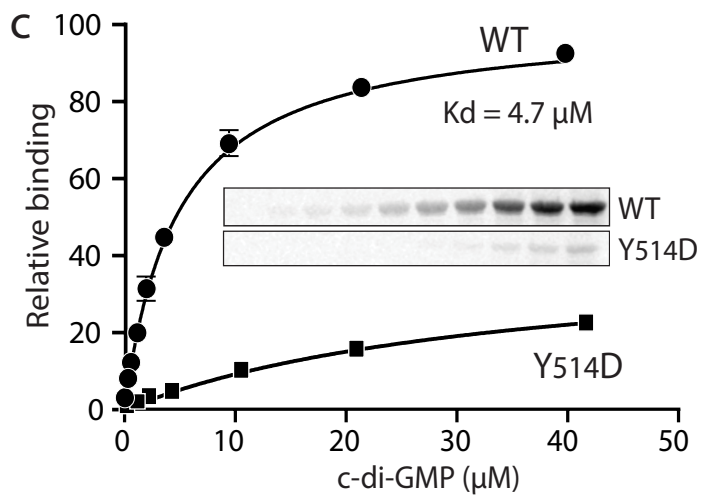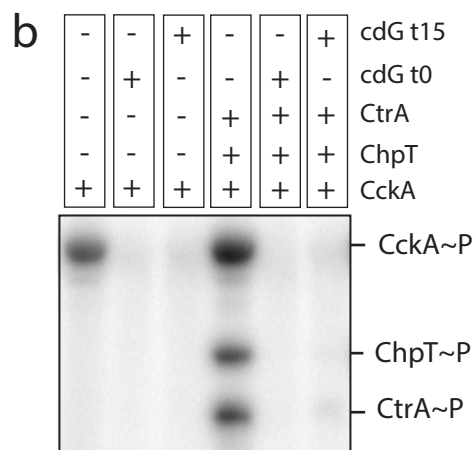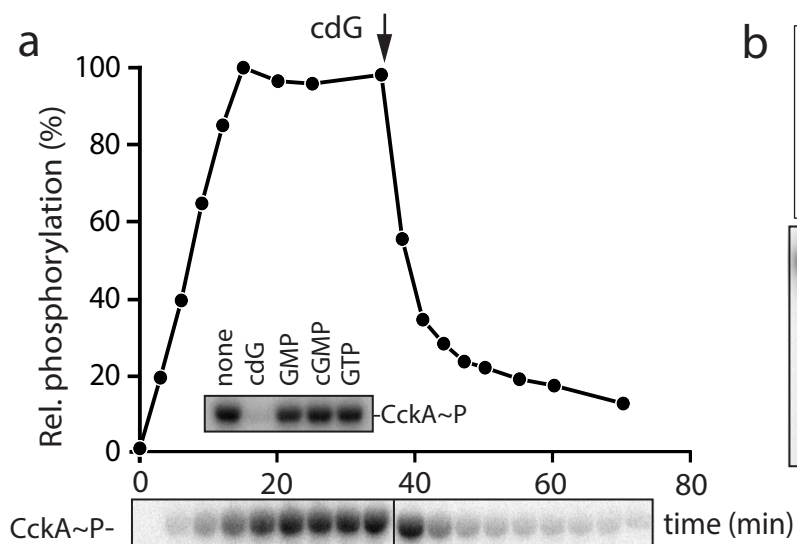
11 tion via PopA and the ClpXP protease.

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Figure 1
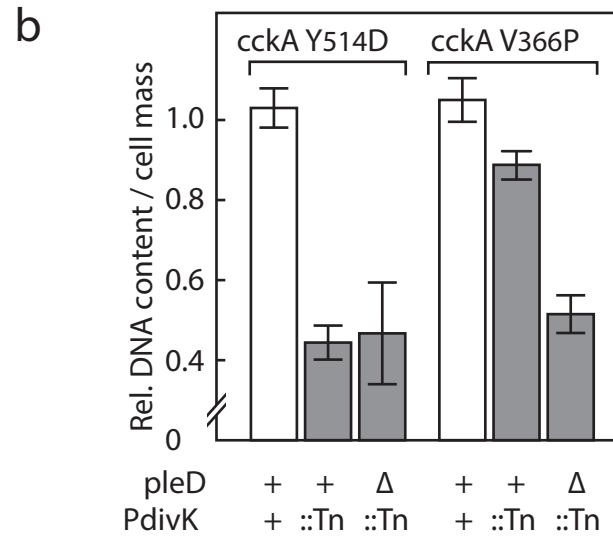
Figure 2

Figure 3

a

cckA	pleD	PdivK	Serial dilutions
WT	+	+	
	+	::Tn	
	Δ	::Tn	
Y514D	+	+	
	+	::Tn	
	Δ	::Tn	
V366P	+	+	
	+	::Tn	
	Δ	::Tn	

b

	cckA Y514D			cckA V366P		
pleD	+	+	Δ	+	+	Δ
PdivK	+	::Tn	::Tn	+	::Tn	::Tn

Figure 4

Swarmer cell/pole

Module1
Phosphatase

Module2
Kinase

PleC —H~P

P i

D—DivK

D—PleD

DivL

CckA —H~P ← ATP
—D~P

ChpT —H~P

CtrA —D~P

⊥

replication

Stalked cell/pole

Module1
Kinase

Module2
Phosphatase

DivJ —H~P

ATP

P~D—PleD

P~D-

DivL

DivK

cdG

CckA —H~P
—D~P

P i

ChpT —H~P

CtrA —D~P

PopA

ClpXP