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ParB-Stimulated Nucleotide Exchange Regulates a Switch in Functionally Distinct ParA Activities

Jesse Easter, Jr., and James W. Gober[1]
Department of Chemistry and Biochemistry and the Molecular Biology Institute
University of California, Los Angeles
Los Angeles, California 90095

Summary

ParA and ParB of *Caulobacter crescentus* belong to a conserved family of bacterial proteins implicated in chromosome segregation. ParB binds to DNA sequences adjacent to the origin of replication and localizes to opposite cell poles shortly following the initiation of DNA replication. ParA has homology to a conserved and widespread family of ATPases. Here, we show that ParB regulates the ParA ATPase activity by promoting nucleotide exchange in a fashion reminiscent of the exchange factors of eukaryotic G proteins. Furthermore, we demonstrate that ADP-bound ParA binds single-stranded DNA, whereas the ATP-bound form dissociates ParB from its DNA binding sites. Increasing the fraction of ParA-ADP in the cell inhibits cell division, suggesting that this simple nucleotide switch may regulate cytokinesis.

Introduction

How newly replicated bacterial chromosomes segregate or partition toward opposite poles of the predivisional cell is not well understood. Chromosomally encoded homologs of plasmid partitioning proteins, ParA and ParB, may function to orient the origin of replication regions (*oriC*) toward the poles (Sharpe and Errington, 1996; Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997; Mohl and Gober, 1997). These proteins in plasmids, such as F factor (SopA and SopB) and bacteriophage P1 (ParA and ParB), which in the prophage form exists as a circular episome, are required for faithful DNA segregation (Gordon and Wright, 2000). SopB and ParB are sequence-specific DNA binding proteins that bind to a centromere-like sequence in these plasmids called *sopC* or *parS*. ParA (SopA) is an ATPase and possesses a conserved helix-turn-helix sequence that binds to an operator sequence upstream of the *parAB* (*sopAB*) operon in these plasmids and functions as a transcriptional repressor (Gordon and Wright, 2000). In vitro experiments have shown that the nucleotide-bound state of P1 ParA dictates whether it binds to operator sequences and represses transcription or interacts with the ParB-*parS* partitioning complex (Davey and Funnell, 1997; Bouet and Funnell, 1999; Fung et al., 2001). ParB itself may regulate this switch in activities, since ParB has been demonstrated to modestly stimulate ParA-catalyzed ATP hydrolysis (Davis et al., 1992; Davey and Funnell, 1994).

The chromosomally encoded homologs of these plasmid partitioning proteins have been identified in at least 29 eubacterial genomes (Yamaichi and Niki, 2000). In the sporulating bacterium, *Bacillus subtilis*, the *parB* homolog, *spo0J*, is required for the expression of class II sporulation promoters (*spoII*) (Ireton et al., 1994). Mutations in the *parA* homolog, *soj*, were shown to relieve the sporulation defect in a *spo0J* mutant strain. In vivo crosslinking experiments have shown that Spo0J binds to multiple sites adjacent to *oriC* (Lin and Grossman, 1998). Subcellular localization experiments using either GFP fusions or immunofluorescence microscopy have shown that Spo0J is found at two discrete foci near the poles of the cell in a pattern that mimics the location of *oriC* (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997; Webb et al., 1998; Sharpe and Errington, 1998). One hypothesis is that Spo0J is bound to *oriC* in order to signal the completion of partitioning to the sporulation pathway. Soj functions to repress class *spoII* promoters in the absence of Spo0J and/or partitioning. In this regard, it has been demonstrated that Soj represses transcription by binding to RNA polymerase-catalyzed melted regions of class *spoII* promoters (Cervin et al., 1998), and in vivo crosslinking experiments have shown that Soj binds to several early sporulation promoters (Quisel et al., 1999; Quisel and Grossman, 2000).

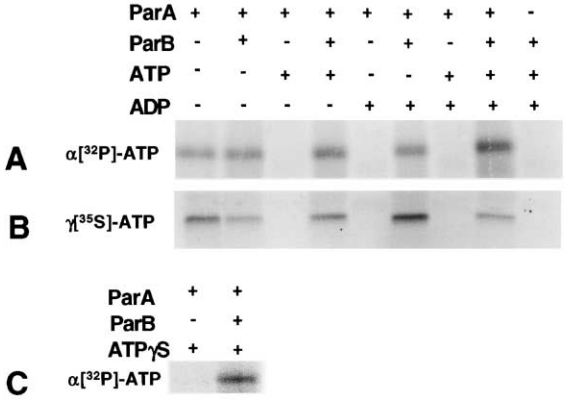
In the dimorphic bacterium, *Caulobacter crescentus*, *parA* and *parB* are essential for viability (Mohl and Gober, 1997; Mohl et al., 2001). ParB has been shown to bind to sequences adjacent to the origin of replication and localize to the poles of the predivisional cell (Mohl and Gober, 1997). Depletion of ParB or overexpression of ParA results in a severe cell division defect with the formation of filamentous cells that lack FtsZ rings (Mohl et al., 2001), indicating that ParA and ParB regulate cytokinesis. Here, we show that ParA can stably exist in an ADP- or ATP-bound state and that ParB can regulate this switch by promoting nucleotide exchange. The two different nucleotide-bound forms of ParA differ in their ability to bind DNA and influence ParB-DNA interaction. ParB-mediated nucleotide exchange is probably critical for cytokinesis, as overexpression of ParA results in an increase in the relative fraction bound to ADP and an inhibition of cell division.

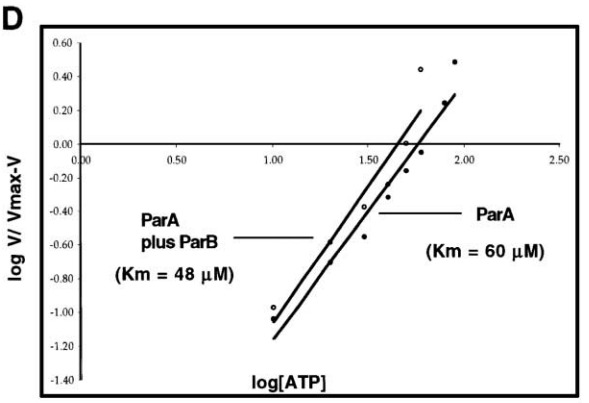
Results and Discussion

ParB Regulates ParA-Catalyzed ATP Hydrolysis by Stimulating Nucleotide Exchange

We found that depletion of ParB from *C. crescentus* resulted in a marked cell division defect and hypothesized that ParA was inhibiting cell division in the absence of ParB (Mohl et al., 2001). Experiments with ParA from bacteriophage P1 have shown that ADP-bound ParA functions as a repressor and that the ATP-bound form interacts with ParB bound to *parS* sequences (Bouet and Funnell, 1999). Therefore, we wanted to investigate whether *C. crescentus* ParB could regulate ParA by influencing its nucleotide-bound state. We first tested

[1]Correspondence: gober@chem.ucla.edu





E

ATP

	Rate (M/sec)	Turnover # (1/sec)	Fold Stimulation	Molecules/minute	Minutes to hydrolyze 1 molecule of ATP
ParA	7.9E-10	1.3E-02	1	0.79	1.3
ParA + ParB	3.7E-09	6.2E-02	5	3.70	0.3

ADP

	Rate (M/sec)	Turnover # (1/sec)	Fold Stimulation	Molecules/minute	Minutes to hydrolyze 1 molecule of ATP
ParA	4.0E-11	6.7E-04	1	0.04	25.0
ParA + ParB	3.4E-09	5.7E-02	86	3.43	0.3

F

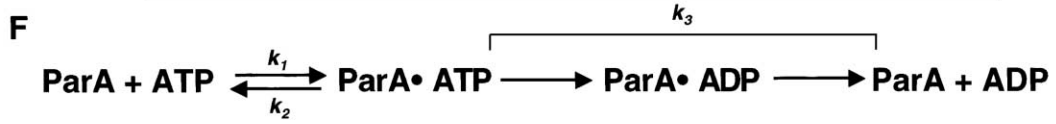


Figure 1. ParB Functions as a Nucleotide Exchange Factor for the ParA ATPase

(A) Purified ATP-bound ParA (1.8 μM, lanes 1–8) was preincubated (30 s, 4°C) with nonradioactive nucleotides (100 μM) as indicated and chased with 500 nM [α-^{32}P]ATP (A) and ParB (1.8 μM, lanes 2, 4, 6, 8, and 9). The samples were UV-crosslinked for 30 s at 4°C, subjected to SDS-PAGE, and analyzed by autoradiography.

(B) The experiment was repeated as before except that the slowly hydrolyzable analog [γ-^{35}S]ATP (1.2 μM) was used for the chase. For both experiments, lane 9 contained all the reaction components except ParA.

(C) The experiment was repeated as in (B) except that ParA was preincubated in nonradioactive ATPγS (100 μM) and 500 nM [α-^{32}P]ATP was used for the chase.

(D) ParA (300 nM) was incubated for 30 s at 30°C with increasing concentrations of ATP (0–100 μM) in the presence or absence of ParB (300 nM). A function of the initial rate of ATP hydrolysis is plotted versus the log of the ATP concentration (Hill plot) in the presence and absence of equimolar ParB. The standard deviation from three independent experiments was less than 3.1%.

(E) Kinetic data for ParA-catalyzed ATP hydrolysis reactions at saturating substrate concentration (100 μM). Shown is a comparison of ParA purified in the presence of ATP or ADP and incubated with or without ParB. All assays were conducted three separately purified ParA preparations (standard deviation less than 3.0% without ParB and less than 2.5% with ParB).

(F) Kinetic model of ParA-catalyzed ATP hydrolysis. The data presented suggest that ParB stimulates the catalytic rate constant (k_3) through the enhancement of nucleotide exchange. This rate is a composite of the intrinsic hydrolysis rate plus the rate of release of product (ADP).

whether ParB could stimulate the exchange of ParA-bound nucleotide with nucleotide in solution (Figure 1A). When ParA was incubated in buffer with γ-[^{32}P]-ATP and no additional nucleotides present, there was some binding of nucleotide (Figure 1A, lane 1). Note that the magnitude of binding here is low because the ATP concentration (0.5 μM) is well below the experimentally determined Km for ATP (approximately 50 to 60 μM) (see Figure 1D). The inclusion of ParB in this reaction did not influence the amount of bound, radioactive nucleotide (Figure 1A, lane 2). In contrast, when ParA was preincubated with either nonradioactive ATP (100 μM) (Figure 1A, lane 3), ADP (100 μM) (Figure 1A, lane 5), or both nucleotides (Figure 1A, lane 7) and followed by a chase of γ-[^{32}P]-ATP, there was no subsequent binding of radioactive nucleotide. The inclusion of ParB in these reactions, however, resulted in a rapid exchange of γ-[^{32}P]-ATP in solution for nonradioactive ParA-bound nucleotide (Figure 1A, lanes 4, 6, and 8). When ParA was preincubated with either ATP or ADP and then the reaction was chased with γ-[^{35}S]-ATP, similar results were obtained; ParA was unable to bind to γ-[^{35}S]-ATP (Figure 1B, lanes 3, 5, and 7) unless ParB was included in the reaction mixture (Figure 1B, lanes 4, 6, and 8). We then repeated this experiment by preincubating ParA with nonradioactive ATPγS and chasing with γ-[^{32}P]-ATP. Once again, exchange did not occur unless ParB was included in the reaction mixture (Figure 1C). Thus, ParB-stimulated exchange did not require ATP hydrolysis. These results indicate that ParB functions as a nucleo-

tide exchange factor for ParA. ParB was equally capable of stimulating the exchange of ADP or ATP. We hypothesize that interaction with ParB stabilizes the nucleotide-free form of ParA, thus releasing either ATP or ADP. This would be analogous to the mechanism whereby the nucleotide exchange factors SOS and CDC25 stimulate GDP-GTP exchange in Ras (Boguski and McCormick, 1993).

Next, we wished to determine whether *C. crescentus* ParA-catalyzed hydrolysis of ATP was influenced by ParB. Purified active ParA was obtained only if ATP or ADP was included during the purification (see Experimental Procedures) (data not shown). In the experiments presented here, we used ParA that had been purified in the presence of ATP. We first assayed the rate of ParA-catalyzed ATP hydrolysis in the presence and absence of ParB (Figure 1D). These assays were all conducted under initial rate conditions with varying concentrations of substrate. A plot of substrate concentration versus rate of ADP formation revealed sigmoidal kinetics (not shown), indicating that ATP hydrolysis was subject to cooperativity. The addition of ParB to the reaction mixture had no significant effect on the cooperativity (Hill coefficient (n) of 1.58 without versus 1.7 with ParB) or the apparent Km (60 μM versus 48 μM) for ATP (Figure 1D).

The nucleotide exchange experiments predicted that ParB would stimulate ParA-catalyzed ATP hydrolysis by promoting the release of ADP, and thus should have an effect on the catalytic rate constant (k_{cat}). To test this idea, we conducted ATP hydrolysis assays under conditions in which ATP substrate concentrations were saturating (100 μM) (Figure 1E) and compared the rate of product formation using ParA that had been purified in the presence of ATP and in the presence of ADP. In assays that utilized ParA purified in the presence of ATP, the k_{cat} was 0.79 molecules of ATP hydrolyzed per min (Figure 1E). The addition of ParB resulted in a 5-fold stimulation of the k_{cat} (3.7 min^{-1}). An even more dramatic effect on ParA ATPase activity was observed when ParA purified in the presence of ADP was used in the experiment. In this case, the catalytic rate constant increased 86-fold in the presence of ParB (0.04 min^{-1} versus 3.43 min^{-1}) (Figure 1E). In the presence of ParB, the k_{cat} in both cases was almost identical. Interestingly, in the absence of ParB, ParA-ATP exhibited a 19-fold greater rate of ATP hydrolysis than ParA-ADP (Figure 1E). We hypothesize that the slower rate of hydrolysis in the ParA-ADP preparation is probably attributable to the fact that ADP is a competitive inhibitor of the hydrolysis reaction (data not shown). This inhibition would be accentuated in the absence of a nucleotide exchange factor. Taken together, these results suggest that one mechanism by which ParB may regulate ParA ATPase activity is the stimulation of nucleotide exchange.

ParA Single-Stranded DNA Binding Activity Is Regulated by Bound Nucleotide

In *B. subtilis,* the ParA homolog, Soj, has been shown to repress class II sporulation genes by binding to melted regions of DNA within the open transcription complex (Cervin et al., 1998). As a test of whether *C. crescentus* ParA possessed the ability to bind to single-stranded DNA, we conducted surface plasmon resonance experiments (SPR) with a sensor chip that contained immobilized single-stranded DNA. Since the identity of the putative cell division gene that ParA represses is unknown, we used an oligonucleotide representing the top strand of the *gidABparAB* operon promoter region. ParA-ATP was unable to bind to the single-stranded oligonucleotide (Figure 2A), whereas ParA-ADP exhibited significant binding (Figure 2B). ParA-ADP could bind to single-stranded DNA both on the chip and in solution. When we pre-mixed ParA-ADP with oligonucleotide at a concentration that was either one-half or equimolar to that bound to the sensor chip, it resulted in a proportional decrease in the amount of ParA-ADP bound to the chip (Figure 2B). The ability of ParA-ADP to bind to the single-stranded immobilized oligonucleotide DNA was completely abolished by preincubating with equimolar amounts of four different soluble competitor single-stranded oligonucleotides representing sequences from other regions of the *C. crescentus* genome (Figure 2C) (see Experimental Procedures), suggesting that the single-stranded DNA binding activity was nonspecific. We also found that neither the addition of ATP (Figure 2D) or ParB (Figure 2F) alone eliminated oligonucleotide binding of ParA prebound with ADP. However, if ATP and ParB were both added to ParA-ADP, no DNA binding was observed (Figure 2G). These experiments indicate that the ParA single-stranded DNA binding activity is regulated by ParB through modulation of the nucleotide-bound state. Interestingly, the observed lack of binding specificity suggests that if ParA-ADP functions as a transcriptional repressor in the same fashion as the *B. subtilis* homolog, Soj, then promoter specificity may be conferred through interaction with another protein.

ParB-*parS* DNA Complex Formation Depends on the Nucleotide-Bound State of ParA

Experiments with ParA from bacteriophage P1 have demonstrated that ATP-bound ParA can stably interact with the partitioning complex, whereas ADP-bound ParA cannot (Bouet and Funnell, 1999). We tested whether *C. crescentus* ParA could interact with ParB in an SPR experiment using an immobilized double-stranded oligonucleotide containing the ParB binding site within the *gidABparAB* promoter region (J.E., R. Figge, and J.W.G., unpublished data). Purified ParB bound to the immobilized DNA with a $t_{1/2}$ of 21 min, indicative of a specific interaction (Figure 3A). Neither ParA-ATP or ParA-ADP bound to this double-stranded oligonucleotide (see Figures 3C and 3D, data not shown). We premixed ParB and either ADP- or ATP-bound ParA and applied this to the immobilized DNA. The addition of equimolar ParA-ADP had no effect on the magnitude of response units (RU) generated in the SPR experiment (Figure 3B), indicating that ParA-ADP did not bind to the ParB-DNA complex. In contrast, when ParA-ATP was premixed with ParB and applied to the sensor chip, no binding of either ParB or ParA was observed (Figure 3C), indicating that ParA-ATP prevented ParB from associating with the *parS* binding site. We next mixed a fixed concentration of ParB with differing amounts of ParA-ATP. Interestingly, the number of ParB molecules bound to the sensor chip in this experiment was inversely proportional to the concentration of ParA-ATP (Figure 3D), indi-

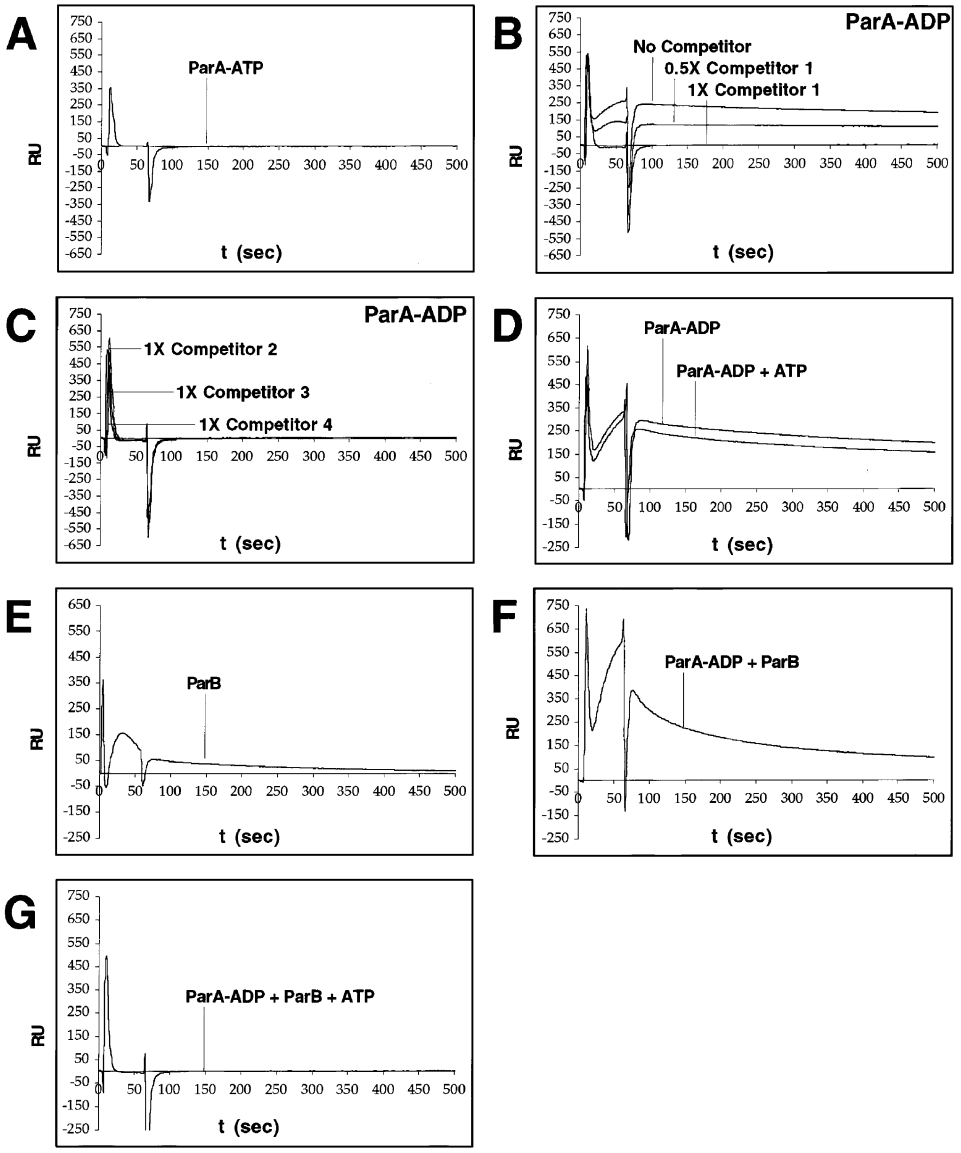


Figure 2. ParB Regulates ParA Single-Stranded DNA Binding Activity by Nucleotide Exchange

A biotinylated, 26 nt single-stranded oligonucleotide containing sequences from the *gidABparAB* operon promoter region was immobilized on a streptavidin sensor chip and used in surface plasmon resonance (SPR) experiments to determine if ParA binds to single-stranded DNA (ssDNA). A typical sensorgram is shown for injections of: (A) ATP-bound ParA; (B) ADP-bound ParA premixed with 0, 375 nM, or 750 nM of the same ssDNA as that immobilized to the sensor chip as a competitor; (C) ADP-bound ParA mixed with 750 nM of 3 random ssDNA competitors; (D) ADP-bound ParA and ADP-bound ParA premixed with ATP; (E) ParB control; (F) ADP-bound ParA premixed with ParB; and (G) ADP-bound ParA premixed with ParB and ATP.

cating that ParA-ATP can prevent ParB from binding DNA. We next tested whether ParA-ATP could actively dissociate ParB from its DNA binding site, by first binding ParB to immobilized *parS* DNA. The addition of an equimolar amount of ParA-ATP resulted in the rapid dissociation of ParB from the immobilized DNA (Figure 3F). These results indicate that ParA can influence the formation and/or stability of the partitioning complex.

ParA Influences ParB DNA Binding Activity In Vivo

An unexpected finding from these experiments was the ability of ParA-ATP to dissociate ParB from its DNA binding site. We wanted to test whether ParA could dissociate ParB from its DNA binding site in *C. crescentus* cells. We introduced *parA* under the control of a xylose-inducible promoter on a multi-copy plasmid and tested whether overexpression of *parA* could affect ParB DNA binding as assayed by two different methods. We found that overexpression of a xylose-inducible *parA* could affect ParB-mediated repression of a *gidA-lacZ* transcriptional reporter fusion, causing an approximately 6-fold increase in the rate of β-galactosidase synthesis (Figure 4A), suggesting that ParA was dissociating ParB-DNA complexes at the *gidABparAB* promoter. The biological significance of this activity is unclear. It is possible that dissociation of ParB from

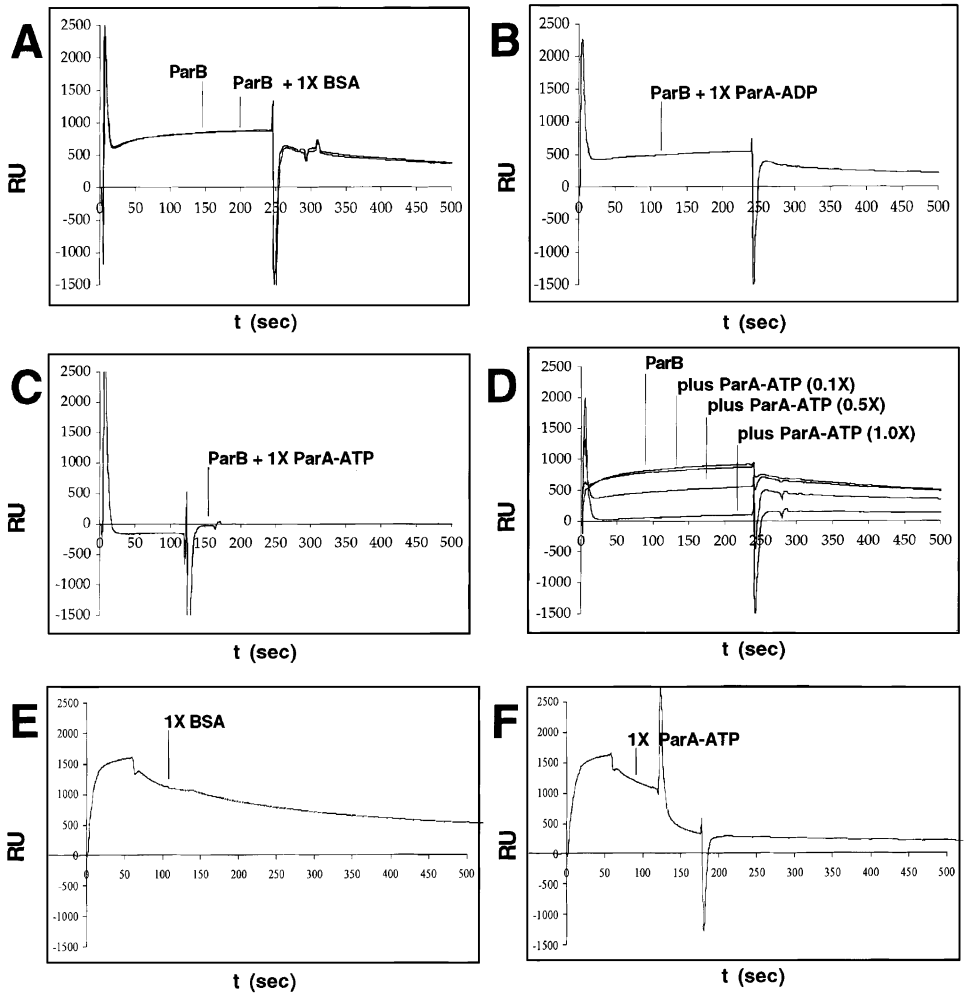


Figure 3. ParB-DNA Complex Formation Is Influenced by the Nucleotide-Bound State of ParA

A biotinylated 26 bp double-stranded oligonucleotide containing sequence of the ParB binding site within the *gidABparAB* operon promoter region was immobilized on a streptavidin sensor chip and used to investigate the effects of ParA on ParB DNA binding activity by SPR. A typical sensorgram is shown for injections of: (A) ParB and ParB with bovine serum albumin; (B) ParB premixed with equimolar ADP-bound ParA; (C) ParB premixed with equimolar ATP-bound ParA; and (D) ParB premixed with 0, 0.15 μM, 0.75 μM, or 1.5 μM ATP-bound ParA. In panels (E) and (F), ParB was prebound to the sensor chip, and 5 μl of 1.5 μM (E) bovine serum albumin (in buffer A) or (F) ParA-ATP was injected.

DNA serves to regulate temporal expression of the *gidABparAB* operon which exhibits peak transcription in late predivisional cells (Mohl and Gober, 1997). We hypothesize that the ParA-ATP-mediated dissociation of ParB from its binding site within the promoter of this operon could relieve repression in the predivisional cell. As an additional test for the ability of ParA to dissociate ParB-DNA complexes in vivo, we assayed for the presence of polar ParB foci using immunofluorescence microscopy in the strain containing the xylose-inducible *parA*. Induction of *parA* expression by the addition of xylose to the growth medium resulted in the gradual appearance of filamentous cells that had lost ParB foci (Figure 4B). This result is consistent with the idea that high intracellular concentrations of ParA abolish ParB DNA binding activity.

Overexpression of *parA* resulted in a cell division block with the accumulation of filamentous cells (Figure 4B). We have previously shown that this block in division is attributable to the inability to complete the first step in cytokinesis: the assembly of FtsZ rings (Mohl et al., 2001). We wanted to test whether this cell division block was caused by a shift in the fraction of ParA bound to ADP. To accomplish this, cultures were grown in the presence of $^{32}PO_4^{-3}$ for approximately five generations, the cells were lysed, ParA-nucleotide complexes were immunoprecipitated, and the bound radioactive nucleotides were quantitated using thin-layer chromatography (TLC). As a control, preimmune sera was used in conjunction with labeled extracts from wild-type cultures. No radioactive nucleotides were recovered in this control experiment (Figure 4C). When anti-ParA antibody was employed, in mid-log-phase wild-type cultures, most of the ParA (85%) was bound to ATP (Figures 4C and 4D). In contrast, when ParA was overexpressed, only 31% was in the ATP-bound form after 6 hr of induction (Figures 4C and 4D). There was no significant decrease in the levels of cellular ATP in these cells (Figure

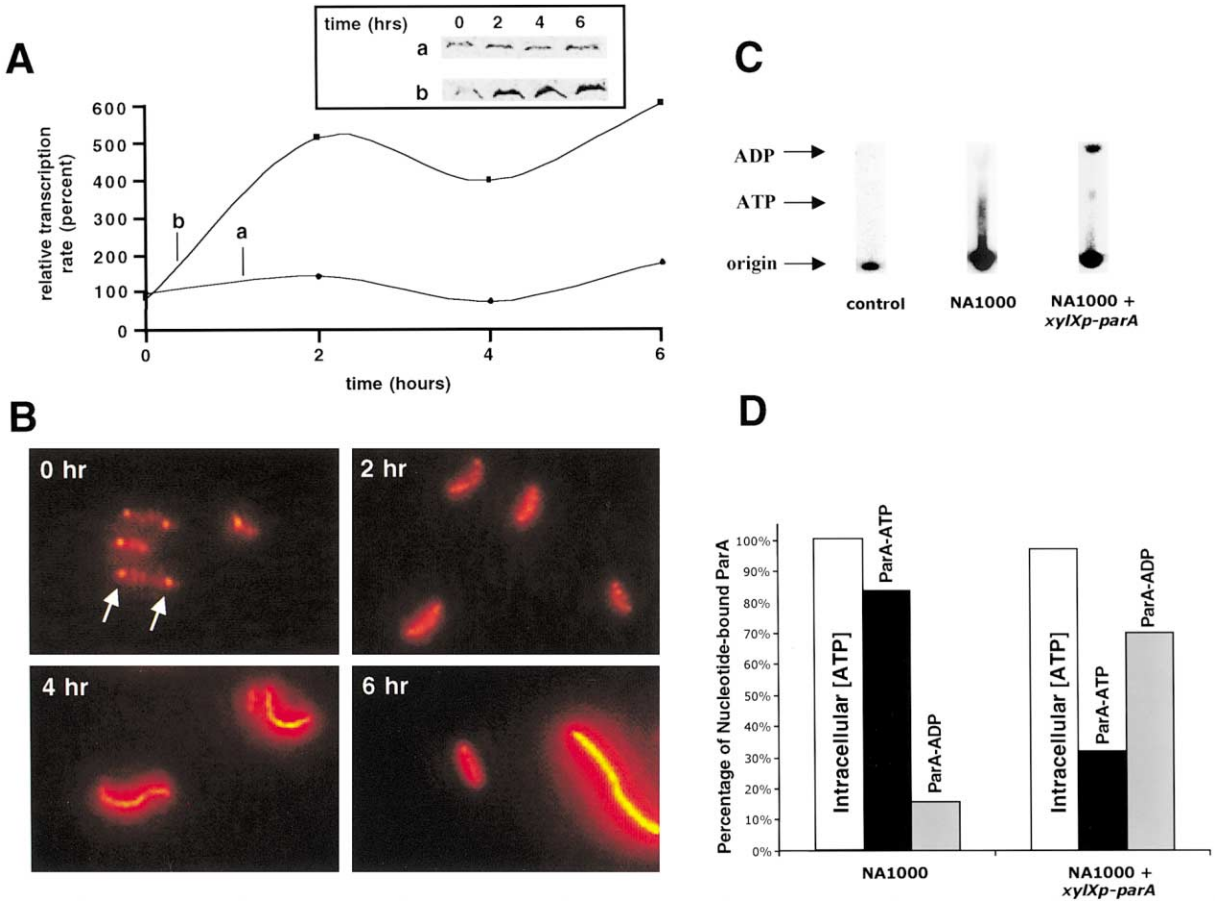


Figure 4. Effect of Increased Intracellular ParA Concentration on ParB DNA Binding Activity and ParA-ATP/ParA-ADP Ratio

(A) Pulse-chase experiments were performed on cultures of *C. crescentus* strain NA1000, carrying *gidA-lacZ* reporter fusion (a) and NA1000 containing the reporter fusion and a multi-copy plasmid with *parA* under the control of a xylose-inducible promoter (b). Xylose was added in order to induce parA expression; a portion of the culture was removed at various times. Proteins were pulse-labeled and β-galactosidase was immunoprecipitated. Plotted is the relative transcription rate (in percent) over time following xylose addition. The inset of the graph shows the data from the phosphorimager.

(B) The subcellular localization of ParB was assayed in *C. crescentus* cells harboring a xylose-inducible *parA*. Portions of the culture were removed at various times following the addition of xylose, and the cells were fixed and processed for immunofluorescence microscopy. Polar ParB foci are only present at time = 0 hr and are indicated by arrows.

(C) Ratio of ParA-ATP/-ADP in mid-log cultures and following ParA overexpression. Cultures of *C. crescentus* either with or without a xylose-inducible *parA* were grown for five generations in low phosphate media to mid-log phase in the presence of $^{32}PO_4^{3-}$. ParA was immunoprecipitated, and radioactive nucleotides recovered from immunoprecipitated ParA were separated by TLC and visualized by autoradiography. A control chromomatogram using pre-immune serum is also shown.

(D) Quantitation of the intracellular ParA-ATP/ADP ratio. The developed nucleotides on the TLC plates shown in (C) were quantitated for the amount of radioactivity present by phosphorimager analysis and compared to the intracellular ATP levels. The percentages represent the mean values from at least three different experiments. The standard deviation in all cases was less than 4.0%.

4D). This result indicates that ParA requires a critical concentration of ParB in order to switch to an ATP-bound form and provides evidence that ParA-ADP may function to inhibit cell division.

Conclusions

ParA proteins belong to a family of ATPases which include MinD, the bacterial topological specificity factor for cell division, and the more distantly related, nitrogenase iron protein (Fe-protein) (Motellebi-Veshareh et al., 1990; Koonin, 1993). Both MinD and Fe-protein possess structural similarity with $p21^{ras}$ (Ras) and other signal transducing G proteins in sequences important for nucleotide binding and hydrolysis (Georgiadis et al., 1992; Hayashi et al., 2001). Nitrogenase Fe-protein, like G proteins, switches between two different conformational states depending on whether the NTP or NDP form of the nucleotide is bound (Rees and Howard, 1999). The results presented here show that ParA from *C. crescentus* is regulated by a nucleotide switch, which alters its biochemical properties, suggesting that this may be a general property of this family of ATPases. For example, ATP hydrolysis is required for partitioning function but inhibits DNA repressor activity of ParA from bacteriophage P1 (Fung et al., 2001). The chromosomally encoded "partitioning" proteins in this family are likely to operate a simple signal transduction system that couples the polar localization of newly replicated origin regions to critical cellular processes. We propose that ParA cycles between ADP- and ATP-bound forms and

that the relative fraction of ParA in each state is regulated by ParB, perhaps in response to partitioning. A similar switch in ParA activities probably operates in *B. subtilis*, as mutants of Soj (ParA) defective in ATP binding or hydrolysis were unable to inhibit sporulation in the absence of Spo0J (ParB) (Quisel et al., 1999).

Experimental Procedures

Growth of Cells

Caulobacter crescentus NA1000 and its derivatives were grown at 31°C as previously described (Poindexter, 1964). The rate of *gidABparAB* operon promoter activity was assayed using a *gidA-lacZ* transcription fusion (our unpublished data) on plasmid p*lacZ*/290. The rate of transcription was determined as previously described (Gomes and Shapiro, 1984). Immunofluorescence microscopy with anti-ParB antibody was performed as described (Mohl and Gober, 1997).

Protein Purification and Biochemical Assays

ParB was purified as described in Mohl and Gober (1997) except that PBS was substituted for the final dialysis buffer. Contaminating ATPase activity was removed by purifying His_6-ParB over heparin sepharose and dialyzed against 50 mM HEPES (pH 7.5), 50 mM KCl, 10% (v/v) glycerol, and 1 mM dithiothreitol (DTT). His_6-tagged ParA was purified in essentially the same manner as His_6-tagged ParB except that 30 μM ATP or ADP was added to all buffers and 25 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, 10% (v/v) glycerol, and 1 mM DTT supplemented with 30 μM ATP or ADP was used for the final dialysis.

The rate of ATP hydrolysis was measured by following the conversion of α-^{32}P-ATP to α-^{32}P-ADP over time as described (de Boer et al., 1991). After polyethyleneimine (PEI)-cellulose TLC, ADP and ATP spots were quantified with a phosphorimager (Molecular Dynamics). UV-induced crosslinking of radiolabeled nucleotides to ParA was performed as described (de Boer et al., 1991).

DNA Binding and Interaction Assays

All DNA binding experiments were done by surface plasmon resonance (SPR) on a BIAcore X instrument at 25°C in HBS-P buffer (BIAcore AB, Uppsala, Sweden) at a flow rate of 5 μl/min. The DNA was immobilized on a streptavidin sensor chip by injections of 10 μl of 10 nM DNA for a final yield of 180 response units for single-stranded DNA and 310 response units for double-stranded DNA. A biotinylated oligonucleotide containing the sequence of the top strand of the ParB binding site within the *gidABparAB* operon promoter region (5′-GAGGCTTGTTTCACGTGAAACATCGG [biotinylated at the 5′ end]) (J.E., R. Figge, and J.W.G., unpublished data) was used as the single-stranded DNA substrate for binding assays using ParA. This same oligonucleotide annealed to a second oligonucleotide containing the complementary sequence (5′-CCGATGT TTCACGTGAAACAAGCCTC) was used as a double-stranded substrate DNA for ParB binding assays. All protein injections were 5 μl at concentration of 1.5 μM unless otherwise indicated. The sequences of the single-stranded DNA used in competition experiments were 5′-CTGAAAAAGCTTCAGGAAAGCCACGGC for competitor 1, 5′-AGTAAGAAGCTTGTCGAGCAAGATTACAGGATT for competitor 2, 5′-GGCGCGGATTCCGCTCGCGAAAGTCGGCCG for competitor 3, and 5′-AGGATTCGGCGCGACTTCCGCACGCGAAAG for competitor 4.

Determination of Intracellular Adenine Nucleotide-Bound Forms of ParA

The ratio of ATP- to ADP-bound ParA in vivo was determined essentially as described (Katayama et al., 1998). *C. crescentus* cells were grown in low phosphate M5-glucose medium at 31°C. Exponentially growing cultures were subcultured into 10 ml of fresh medium containing 320 μM K_2HPO_4 and ^{32}P-orthophosphate (0.4 mCi/ml), and the culture was allowed to grow from an OD_{600} of 0.022 to 0.35. The cells were harvested by centrifugation, washed once with 1 ml 0.9% NaCl, and lysed. Preparation of extracts for immunoprecipitation and nucleotide analysis was described in Katayama et al. (1998). Radioactive ADP and ATP was quantified from phosphorimages of the TLC plate by selecting the area of the spot with the peak of radioactivity. Intracellular ATP concentrations were determined (Otto et al., 1984) using a Sigma-Aldrich Bioluminescence Kit.

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