Dual Role of DNA in Regulating ATP Hydrolysis by the SopA Partition Protein*1, 2

Received for publication, July 15, 2009, and in revised form, September 8, 2009. Published, JBC Papers in Press, September 8, 2009, DOI 10.1074/jbc.M109.044800

Yoan Ah-Seng15, Frederic Lopez3, Franck Pasta15, David Lane1, and Jean-Yves Bouet35,2

From the 1Laboratoire de Microbiologie et Génétique Moléculaires, CNRS, F-31000 Toulouse, France, the 2Laboratoire de Microbiologie et Génétique Moléculaires, Université Paul Sabatier, F-31000 Toulouse, France, and the 3Institut L. Bignard-INSM-CHU Rangueil, F-31000 Toulouse, France

In bacteria, mitotic stability of plasmids and many chromosomes depends on replicon-specific systems, which comprise a centromere, a centromere-binding protein and an ATPase. Dynamic self-assembly of the ATPase appears to enable active partition of replicon copies into cell-halves, but for Walker-box partition ATPases the molecular mechanism is unknown. ATPase activity appears to be essential for this process. DNA and centromere-binding proteins are known to stimulate the ATPase activity but molecular details of the stimulation mechanism have not been reported. We have investigated the interactions which stimulate ATP hydrolysis by the SopA partition ATPase of plasmid F. By using SopA and SopB proteins deficient in DNA binding, we have found that the intrinsic ability of SopA to hydrolyze ATP requires direct DNA binding by SopA but not by SopB. Our results show that two independent interactions of SopA act in synergy to stimulate its ATPase. SopA must interact with (i) DNA, through its ATP-dependent nonspecific DNA binding domain and (ii) SopB, which we show here to provide an arginine-finger motif. In addition, the latter interaction stimulates ATPase maximally when SopB is part of the partition complex. Hence, our data demonstrate that DNA acts on SopA in two ways, directly as nonspecific DNA and through SopB as centromeric DNA, to fully activate SopA ATP hydrolysis.

Faithful segregation of low copy number plasmids in bacteria depends on partition loci, named Par. Such loci are composed of two genes, generically termed parA and parB, encoding an ATPase and a DNA-binding protein, respectively, and a cis-acting centromeric site parS (reviewed in Ref. 1). These three essential elements are sufficient for the partition process. ParBs assemble on parS to form nucleoprotein structures called partition complexes (2–6). ParA ATPases are considered to be motors that direct displacement and positioning of partition complexes inside the cell.

Partition systems have been classified into two major types, distinguished by the nature of their ATPase proteins (7). Type I is characterized by Walker box ATPases, which are specified by many plasmids and most bacterial chromosomes. In some (Type Ia) the nucleotide-binding P-loop is preceded by an N-terminal regulatory domain, in the others (Type Ib) it is not. Type II specifies actin-like ATPases and is present on relatively few plasmids. It is presently the best understood system at the molecular level (8–10). However, the underlying mechanism that drives partition still remains elusive for both systems. Our work aims at the understanding of an archetypal representative of Type Ia, namely SopABC of the Escherichia coli plasmid F.

The several activities of Type Ia ParA proteins are regulated by binding of adenine nucleotides (11, 12), which induce conformational changes in the proteins (13, 14). In their apo and/or ADP-bound forms these proteins display site-specific DNA binding activity, recognizing their cognate promoters through their N-terminal domains. Such activity is involved in the auto-regulation of par operon expression (15, 16). In the ATP-bound form, they specifically interact with cognate partition complexes through contact with ParB proteins. The ATP-bound form of type I ParAs spontaneously forms polymers, which appear as bundled filaments in electron micrographs (12, 17–19). The role of these filaments is not understood but they could be related to the rapid movement of partition complexes in the cell. In vivo, ParA proteins form dynamic assemblies that move back and forth in the cell if the cognate ParB protein and parS centromere are present (20–23). The link between this oscillatory behavior and the segregation of partition complexes is not clear. They both require the ATPase activity of ParA proteins but the role of ATP hydrolysis in the partition process is not understood.

It has long been known that ParA partition proteins exhibit low intrinsic ATPase activity (24, 25). ATP hydrolysis is modestly stimulated by either DNA or the cognate ParB alone but is strongly activated (up to 35-fold) when both DNA and ParBs are present (12, 24, 25). The lack of major stimulation of ATPase by DNA in the absence of ParB proteins has been taken to mean that the DNA-bound form of ParB is the effective activator (26). However, incorporation of centromere sites in the DNA added to ParB did not increase stimulation of ATPase (24, 25), leaving doubts as to the role of the partition complex in ATPase activation.

The mechanism by which ATP hydrolysis acts in the partition process is not known for type I systems. This is in marked contrast to actin-based partition ATPases whose ATPase activity is stimulated in growing filaments (8), where it provokes the rapid disassembly of filaments unless these are capped by the
Partition ATPase Activation by Partition Complex and DNA
cognate partition complex (9). Therefore, for the type II partition system, ATP hydrolysis ensures discrimination between unproductive filaments that are rapidly disassembled and productive filaments that drive partition complexes to opposite ends of the cell. This dynamic instability, which ensures elongation of actin-like filaments only between two partition complexes to be segregated, thus provides regulation of the partition process.

Recently, it has been shown that two members of the type I ParA family, Soj of *Thermus thermophilus* and SopA of plasmid F, bind nonspecific DNA in the presence of ATP (12, 26). Two studies revealed that this DNA binding activity is essential for partition (27, 28). Importantly, it has been shown that a SopA mutant deficient in DNA binding no longer stimulates ATP hydrolysis efficiently, suggesting that DNA could play a direct role in the regulation of the ATPase activity (28). This finding raises the issue of the interactions required for activation of the type I partition ATPase activity by cognate proteins and DNA.

In this study, we have investigated the mechanism of activation of ATP hydrolysis by SopA. First, we have found that the formation of the F partition complex is required for strong stimulation of the SopA intrinsic ATPase activity. We have also found that the partition complex and DNA stimulate ATP hydrolysis independently but that these two independent interactions act in synergy to amplify SopA ATPase activity. Lastly, we have identified an arginine finger motif in SopB responsible for the stimulation of SopA ATPase activity.

**EXPERIMENTAL PROCEDURES**

*Plasmids and sopB Mutagenesis—*Plasmids used are listed in supplemental Table S3). pJYB57 was made by inserting the *sopC* sequence, amplified by PCR from pDAG114 (29), into pBSK(+), using EcoRV and EcoRI restriction sites. pYAS6 is a pTYB1 derivative (NEBioLabs) into which, the *sopB-G324* gene, PCR amplified from pDAG170 (30), was inserted using NdeI and Sapl restriction sites. *sopB* mutations were introduced into pYAS6 by mutagenic primer-directed replication using the Stratagene QuikChange kit and were verified by sequencing.

Protein Purification—Native SopA protein was purified as described (12). Intein-tagged SopB-G324 and mutant proteins were purified from strain C2833 (NEBioLabs) as follows: overexpression, cell lysis, and purification by affinity chromatography on a chitin column. The protein was then quickly eluted at 30 °C for 4 h or, for SopB*1/*2/*3, at 20 °C for 16–20 h. Cells were lysed by sonication in B buffer (20 mM Hepes-KOH pH 7.8, 500 mM NaCl, 1 mM EDTA) containing 10 μg/ml lysozyme. The lysate was loaded onto a 5–ml chitin column and washed successively with 15 ml of B buffer, 50 ml of 1 M NaCl in B buffer, and 15 ml of B buffer. The column was then quickly eluted with 15 ml of 50 mM dithiothreitol in B buffer, and stored at 4 °C for 16–20 h to allow protein cleavage. After elution, proteins were dialyzed against S buffer (12), loaded onto a mono-S column (Amersham Biosciences) and eluted with a 50–600 mM KCl gradient in S buffer. SopB fractions were kept at −80 °C. SopB-N52 was purified as an intein-tagged peptide as above, except that the final mono-S step was replaced by an amicon ultra 15–30K concentration step, and the flow-through was concentrated on a Vivapin 6K filter device (Sartorius). SopB-N52 was quantified by NMR.

ATPase and Electromobility Shift Assays—ATPase assays and electrophoretic mobility shift assays (EMSA) were performed essentially as described (12). Reaction mixtures for ATPase assays were incubated at 37 °C for 4 h. Chromatographs were developed with 1 M formic acid/0.5 M LiCl. EMSA were performed using 1 nm radiolabeled DNA probes, and binding reaction products were resolved at 4 °C by electrophoresis on 6% polyacrylamide gels in TGE (25 mM Tris base, 190 mM glycine, 10 mM EDTA) for 4 h at 180 V.

Surface Plasmon Resonance Assays—Binding and kinetic studies were performed using a Biacore 3000. Immobilization steps of biotinylated DNA probes, performed on a streptavidin-coated sensorchip in HBS-EP buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20), were stopped when 498 RU of 136-bp DNA probe (reference) and 503 RU of 136-bp *sopC* DNA probe were obtained. Binding analyses were performed with multiple injections of different protein concentrations at 25 °C. Samples, diluted in BD buffer (20 mM Hepes-KOH pH 7.4, 100 mM KCl, 10 mM MgCl₂, 50 μM thiolated salmon sperm DNA, 1 mM dithiothreitol), were injected at 10 μl/min over both sensor surfaces at the same time for 180 s. Reference sensorgrams were subtracted from sensorgrams obtained with the 136-bp *sopC* DNA probe to yield true binding responses. Kinetic constants were calculated using BIAevaluation 4.0.1 software.

**RESULTS**

Centromeric DNA Is Required for Maximal ATPase Activation—To determine whether DNA could play a direct role in SopA ATPase activity, we first standardized our ATP hydrolysis assay. The results in Fig. 1A (left panel) show that the intrinsic SopA ATPase activity (0.3 mol/h/mol) is moderately activated both by DNA (2-fold) and by SopB (~3-fold) and is highly activated (43-fold) when nonspecific DNA (NS DNA) and SopB are added together. These results are equivalent to those obtained previously (12, 24, 25). However, in contrast to previous reports that ATPase stimulation was indifferent to the presence of the cognate centromere (24, 25), we found that use of *sopC*-containing DNA with SopB raised the level of ATPase activation to 122-fold (36 mol/h/mol), 3 times higher than with NS DNA (Fig. 1A). Without SopB, such activation does not occur. The disparity between this and the earlier observations motivated us to clarify the “centromeric effect” observed in our conditions.

We assayed SopA ATPase activity in the presence of SopB as a function of DNA concentration (Fig. 1B). ATP hydrolysis increased in proportion to DNA concentration with both NS and *sopC*-containing DNA, reaching a plateau of maximal activation around 80 μmol/g. At this saturating DNA concentration, *sopC*-containing DNA still activates ATP hydrolysis to a 2.5-fold higher level than NS DNA, indicating that centromeric DNA is needed for maximal ATPase stimulation in our assay conditions.

---

3 The abbreviations used are: EMSA, electromobility shift assay; hth, helix turn helix; NS-DNA, nonspecific DNA; R-finger, arginine finger; SPR, surface plasmon resonance; RU, resonance unit; wt, wild type.
Partition ATPase Activation by Partition Complex and DNA

We then measured SopA ATP hydrolysis as a function of SopB concentration (Fig. 1C). ATPase activity rose sharply with increasing SopB concentration, reaching maximal activation at 6 μM. At this activation plateau ATP hydrolysis is still 35–40% higher with sopC-containing DNA than with NS DNA. At lower SopB concentrations, centromeric DNA is a much better ATPase activator than NS DNA, and sopC-containing DNA activates SopA ATPase 6 times more strongly than NS DNA. Thus, the lower the concentration of SopB the more important sopC becomes for efficient activation of SopA ATPase. Taken together, these observations suggest strongly that the F partition complex, SopB bound to sopC centromere, is required for full activation of SopA ATPase activity, and reinforce the idea that the DNA-bound form of SopB is the effective activator.

**Diminished ATPase Activation of a SopA DNA Binding Mutant**—To examine the influence of DNA on SopA ATP hydrolysis we took advantage of a recently identified SopA mutant, SopA^K340A, impaired in ATP-dependent DNA-binding but still able to interact with SopB (28). Like wt, its basal ATPase activity was stimulated -2-fold by SopB (Fig. 1A, right panel). In contrast to wt SopA however, no stimulation was observed in the presence of DNA. Moreover, addition of NS DNA in the presence of SopB results in only weak activation (3-fold), 14-times less than with wt SopA, confirming that the SopA^K340A DNA binding mutant is severely impaired in ATPase activation (28). When sopC-containing DNA was present along with SopB, SopA^K340A ATPase activity increased only 15-fold, much less than the 122-fold observed with wt SopA. These data indicate that in conditions supporting maximal stimulation of wt SopA, SopA^K340A ATPase is not activated by DNA other than indirectly through SopB.

Because interaction of SopA^K340A with SopB is unimpaired (28), the strong reduction in ATPase stimulation stems from the protein DNA binding deficiency, indicating direct involvement of DNA in activation of SopA ATPase activity. Hence the view that DNA stimulates SopA ATP hydrolysis by binding to SopB appeared too simple, and the question of whether DNA stimulates SopA ATPase independently or through SopB remained open.

**Characterization of SopB DNA Binding Mutants**—To determine whether DNA can also stimulate SopA ATPase via SopB, we constructed SopB mutants deficient in DNA binding. SopB recognizes sopC through a helix-turn-helix (hth) motif comprising residues 178 to 198. SopB^*1, mutated in the first helix (A183P) is expected to lose DNA binding activity and SopB^*2, mutated in the recognition helix (K191A-R195A) is expected to lose sopC-specific binding. Binding of the purified SopBs to specific (sopC) and nonspecific (NS) DNAs in the presence and absence of competitor DNA was analyzed by EMSA (Fig. 2). WT SopB binding is illustrated in Fig. 2A and quantitation of wt and mutant SopB binding is shown in Fig. 2B. To summarize, binding of the SopB mutants conformed to that expected if binding affinity is determined largely by the first helix and specificity by the second. Thus, with competitor present SopB^*1 retained some ability to retard sopC but SopB^*2 did not. When the wt first helix of SopB^*2 is no longer sequestered by competitor its binding to both sopC and NS DNAs exceeds that of SopB^*1 even while the latter continues to bind preferentially to sopC. A third mutant, SopB^*3 (A183D-K191A-R195A), made by combining mutated positions, was both unable to differentiate NS and sopC DNAs and even less active in DNA binding. The distinct DNA-binding character of these three SopB variants enabled us to investigate the interactions required for SopA ATPase activation.

**DNA Can Activate SopA ATPase Independently of SopB**—The three SopB variants were assayed for activation of SopA ATPase activity (Fig. 3). Without DNA, the SopB mutant deficient in sopC-specific binding, SopB^*2, activated ATP hydroly-
sis slightly (1.4-fold), like wt. With either NS or sopC-containing DNA, SopB*2 strongly enhanced ATP hydrolysis, 21- and 26-fold, respectively. The 3-fold stimulatory effect of sopC observed with wt SopB was not seen with SopB*2, consistent with the need for direct SopB-sopC interaction in maximal ATPase activation.

Proteins, SopB*1 and SopB*3, whose mutated Ala-183 position weakens DNA binding, showed an intrinsic ATPase activation ability 3-fold higher than wt. (Fig. 3). In the presence of either NS or sopC-containing DNA they both strongly activated SopA ATPase activity (60–120-fold). Hence, the need for DNA in SopB-mediated stimulation of SopA ATPase is bypassed with these mutants (see last section). The possibility that residual DNA binding by SopB*3 enables this mutant to activate ATPase was excluded by showing that it failed to do so with the SopAK340A DNA-binding mutant (not shown). These data indicate that stimulation of ATP hydrolysis occurs also via direct contact between SopA and DNA, and therefore that two independent interactions are required for SopA ATPase activation, one with SopB and another with DNA.

**SopB Activates SopA ATPase through an Arginine Finger Motif**—Nucleotide hydrolysis by many NTPases is stimulated by an arginine finger (R-finger) motif (31). The R-finger is usu-
ally located in flexible loops, either in cis on the same polypeptide chain or in trans provided by a partner protein, that can enter the catalytic pocket and stabilize the transition state through neutralization of the negative charges generated during phosphoryl transfer. Identification of R-fingers is not possible by direct homology search but a comprehensive survey by

Scheffzek et al. (1998) concluded that they must fulfill the following criteria: (i) they are invariant within a subfamily of GTP/ATP-activating proteins; (ii) they cannot be functionally replaced, even by a basic residue; and (iii) mutations should abrogate stimulatory activity without affecting interaction of the two partner proteins.

The requirement for SopB in ATPase activation suggests that SopB could provide such an R-finger motif in trans. Residues important for specificity of SopB-SopA interaction were shown to reside in the first 42 amino acids of SopB (32). We purified a peptide composed of the first 52 amino acids of SopB, SopB-N52, and assayed its ability to activate SopA ATPase (Fig. 4B). In the absence of DNA, SopB-N52 stimulated basal SopA ATPase activity modestly; at 20 μM, ATP hydrolysis increased ~4-fold. In the presence of DNA, either with or without sopC, ATPase activity was strongly activated by SopB-N52. Maximal activation, reached at 20 μM, was comparable to that obtained with full-length SopB in the presence of DNA (Fig. 1C). Therefore, the SopB ATPase activation domain is present within its first 52 amino acids. Although the SopB-N52 peptide does not carry the hth DNA binding domain, it exhibits NS DNA binding activity at concentrations above 5 μM. However, this NS DNA binding activity is salt-sensitive and is barely detectable above 150 mM KCl (supplemental Fig. S1). By contrast, SopA ATP hydrolysis is still fully stimulated by SopB-N52 in the presence of DNA at 200 mM KCl, where SopB-N52 does not bind DNA. These data confirm that direct interaction of DNA with SopA is important for activation of SopA ATPase.

Three arginine residues are present in SopB-N52 (Fig. 5). We tested two of them, Arg-36 and Arg-42, (excluding the extreme N-terminal one as unlikely) for stimulation of SopA ATPase by mutation to alanine and lysine. The purified mutant SopB proteins were fully functional for binding to sopC DNA (data not shown). SopB(R36A) showed a diminished ability to activate SopA ATPase (Fig. 4A); none in the absence of DNA and a 6-fold reduction in the presence of either NS or sopC DNA compared with wt SopB. Thus, residue Arg-42 is important for ATPase stimulation. However, the other variant, SopB(R42K), activated SopA ATPase activity to the levels obtained with wt SopB, both with and without DNA (Fig. 4A), indicating that although Arg-42 is involved in SopA ATPase activation, it is not an R-finger as defined by Scheffzek et al. (31) (criterion (ii) above).

Neither of the Arg-36 mutants was able to stimulate SopA ATPase in the absence of DNA (Fig. 4A). With NS DNA, SopB(R36A) and SopB(R36K) exhibited 17- and 8-fold reduction, respectively, in SopA ATPase stimulation compared with wt SopB. With sopC-containing DNA, the loss of activity was still high (21-fold) for SopB(R36A) and moderate (5-fold) for SopB(R36K). Thus, in contrast to Arg-42, Arg-36 cannot be fully replaced for SopA ATPase stimulation even by lysine. This strongly suggests that SopB
activated SopA ATPase through the action of Arg-36 as an R-finger. We next checked whether Arg-36 fulfilled the two other criteria.

Arginine 36 Is Conserved within a Small ParB Subfamily—The N-terminal domains of ParB proteins are extremely variable, both in sequence and size, which limits reliable alignment of this domain with the ParB homolog family. To evaluate conservation of the apparent R-finger, we restricted alignment to closely related SopB homologs. We found only nine homologs that have an arginine corresponding to SopB-R36 (Fig. 5). Members of this small subfamily are present on seven plasmids and two phages, which share 45–95% amino acid identity with the whole of the SopB sequence. Arg-42 is not found in all members of this subfamily. It is effectively deleted from the prophage plasmids. In two other members of the group it is replaced by lysine, which, taken with the full stimulation of SopA ATPase by SopB-R42K, argues for a role of this positively charged residue in activation of ATP hydrolysis.

SopB-R36 and -R-42 Variants Interact with ATP-bound SopA—Because the N terminus of SopB includes the SopA recognition domain, attribution of R-finger function to Arg-36 was conditional on the ability of SopB-R36 mutant proteins to interact normally with SopA. We therefore analyzed the interaction properties of the wt and mutant SopB proteins. In EMSA, both Arg-36 mutants were as effective as wt in retarding sopC DNA, showing that their affinity for DNA was unaffected (supplemental Fig. S2). To test interaction of SopB with SopA, we performed surface plasmon resonance (SPR) experiments, in which SopB had been loaded onto immobilized 136-bp DNA probes containing a single SopB binding site (sopC unit -sCu) as shown in Fig. 6A. These dose response data (Fig. 6A), gave a $K_a$ (association) of $2.0 \times 10^5$ mol$^{-1}$ s$^{-1}$, a $K_d$ (dissociation) of $4.6 \times 10^{-4}$ s$^{-1}$ and the resulting $K_D$ of 2.3 nm, comparable with our previous results obtained by EMSA and filter binding experiments (6).

When SopA was injected after formation of SopB/sopC complexes (Fig. 6B), an increase in the SPR signal (about 50 RU) was detected, indicating interaction between SopA and SopB bound to sCu-containing DNA. Such interaction occurred only when ATP was present. If ATP was replaced by ADP or omitted, no interaction was detected. SopA showed no specific binding to sCu DNA, with or without ATP. These results show that SopA in its ATP-bound form interacts with the Sop partition complex. This behavior parallels the ATP-dependent binding of P1 ParA to ParB-parS seen by EMSA (11).
First, the peptide SopB-N52, with no DNA-binding domain, strongly activates SopA ATP hydrolysis (Fig. 4B). This level of activation reaches the highest stimulation observed with wt SopB in the presence of sopC DNA (Fig. 1C). Thus, the R-finger motif present in SopB-N52 is by itself sufficient to ensure maximal activation, provided DNA is present to contact SopA. This suggests that the conformation of full-length SopB imposes a constraint that does not allow the R-finger to efficiently reach the ATP pocket of SopA, and that upon binding to the hth, sopC announces its presence by transmitting a conformational signal to the Nter part of SopB.

Second, analysis of SopB-1 and SopB-3 behavior revealed that they are intrinsically better activators than wt SopB, since in the absence of DNA they both stimulate SopA ATPase activity 3 times more efficiently than wt (Fig. 3), to the same level as achieved by the partition complex. Moreover, in the presence of DNA, these two mutants strongly activate ATPase activity and are insensitive to the presence of sopC. This is particularly evident for the triple mutant, highly impaired for NS and sopC DNA binding activities, which stimulates ATP hydrolysis to the maximal level reached with wt SopB in the presence of sopC. These two DNA binding-deficient SopB mutants have in common a mutation at position Ala-183, which corresponds to a very highly conserved alanine present in the first helix of most subfamilies of hth motifs (33). Mutations of this alanine in SopB could mimic sopC binding and induce a change in SopB conformation similar to that obtained upon SopB binding to sopC. SopB-A183 mutants would then be locked in a stimulatory conformation.

DISCUSSION

ParA proteins must interact with ATP to bring about partition. The observation that binding of Walker-box ParA proteins to nonspecific DNA, itself ATP-dependent, determines their polymerization and partition behavior has refocused attention on the long-known ability of DNA to stimulate ATP hydrolysis (12, 27, 28). Previous data had indicated only that addition of DNA or the cognate ParB protein stimulated ATP hydrolysis slightly and that addition of both (whether or not the DNA had a centromere site) induced a greater-than-additive stimulation of ATPase (24, 25). How DNA acts in this latter amplification, through an independent activation of ParA proteins which is synergistic with that of ParB, or by binding to ParB to convert it to a better stimulator, was unknown. The present study shows that in the case of the Sop system the answer is, both.

Our results effectively define three conditions in which DNA stimulates SopA to hydrolyze ATP, summarized schematically in Fig. 7. Nonspecific DNA alone interacts with the specific binding interface represented by Lys-340 (28), to induce a modest activation of ATPase (Fig. 1A). Nonspecific DNA in the presence of SopB activates SopA ATPase more strongly than either DNA or SopB alone, as seen before, but by using SopB mutants defective in DNA binding we have shown that here also DNA is acting only via its SopA binding interface, not through SopB (Fig. 3). Presumably, changes in conformation or oligomerization induced by the separate contacts DNA and SopB make with SopA mutually reinforce each other to produce a more efficient ATPase. Finally, DNA containing the sopC centromere stimulates maximal ATP hydrolysis by SopA (Fig. 1); this time the additional activation is mediated by SopB (Fig. 3). SopB’s need for sopC to enable it to realize its full stimulatory potential was not observed previously. However, we note the parallel between our data and ParR-mediated stimulation of its cognate ATPase, the actin-like ParM, which is also enhanced by its centromere (34). Our results thus show that DNA is provided in two ways to SopA and SopB form dimers but are represented as unique spheres for simplicity. A, SopA alone exhibits very low intrinsic ATPase activity. B, in the presence of NS DNA, SopA ATPase activity is modestly activated (2-fold). C, interaction with SopB alone stimulates ATP hydrolysis about 3-fold. This activation requires the R-finger motif (curved gray extension entering the SopA catalytic pocket) in the N terminus of SopB. D, when both NS DNA and SopB contact SopA, ATPase activity is highly stimulated (40-fold). These two independent interactions act in synergy. SopA bound to NS DNA could adopt a slightly different conformation that renders the R-finger motif of SopB more efficient for the neutralization of the negative charges generated during phosphoryl transfer. E, in the presence of NS DNA and the partition complex, i.e. SopB bound to sopC-containing DNA (interrupted helices containing a light gray void box), SopA ATPase activity is strongly stimulated (120-fold). As in D, the two independent interactions act in synergy and the 3-fold supplementary stimulation could be provided by a conformational change that would occur upon specific binding to sopC centromere. The R-finger motif of SopB would then adopt an optimal conformation (straight gray extension) in the SopA catalytic pocket for maximal stimulatory activity.
Partition ATPase Activation by Partition Complex and DNA

The identification of two principal mechanisms of ATPase activity clarifies the role of ATP hydrolysis in the functioning of the Sop partition system. Activation of ATPase by DNA alone is probably not significant in vivo. Not only is it weak but in cells carrying the F plasmid SopA is generally accompanied by SopB. The presence of SopB determines two main states of SopA: in one, SopA finds itself close to both DNA and SopB and can hydrolyze ATP to generate an ATP-free form (no-nucleotide/ADP/ADP-P_i), in the other it is shielded from DNA by nonspecifically bound SopB and so retains its ATP. It is ATP-free SopA that binds to the sopAB promoter to enable autoregulation of sopAB transcription (12, 15, 35), and ATPase activation by SopB could account for the corepressor function of this protein that is needed for effective repression. It is the ATP form of SopA that spontaneously forms polymers as long as contact with DNA is avoided (12). The synergistic role of DNA in raising SopB-mediated stimulation of SopA ATPase to effective levels can be viewed as tuning hydrolysis to prepare for the act of partition, by assuring appropriate levels of Sop proteins without obliterating SopA proto-filaments as they wait to seize new partition complexes. In the other state, SopA is in direct contact with the partition complex, resulting in the highest level of ATPase activity. Thus, maximal ATP hydrolysis will be restricted to the region of the centromere(s) where it would be suitably located for displacing partition complexes. Although we have yet to tease out the biochemical details of the displacement mechanism, one factor likely to be important is the change in SopA conformation that ensues upon ATP hydrolysis. The conformation of SopA, like that of other partition ATPases such as P1 ParA, is controlled by adenine nucleotide binding (13, 14). Generation of ATP-free SopA would be particularly intense at the tip of SopA filaments in contact with partition complexes, and the transition to the new conformation could be the trigger for SopA release from them. By analogy to the eukaryotic tubulin involved in mitosis (36), we imagine that this conformational change in the filaments could provide the force to pull partition complexes away from the center of the cell, and thus to drive active partition.

Two other mechanistic aspects of SopB-mediated activation of SopA ATPase have been brought to light. One is the identification of the SopB motif involved in the activation. Mutations of arginine 36 drastically impair stimulation of SopA ATPase activity, despite the undiminished affinity of the mutant proteins for sopC and SopA (Fig. 6C). These are the defining characteristics of an arginine finger. They imply that Arg-36 enters the SopA active site to facilitate processing of the hydrolysis transition state. A functional homologue of SopB belonging to the distinct type Ib class of partition loci, ParG, has also been shown to activate its cognate ATPase, ParF, through an R-finger motif (37), making SopB the second example of such a centromere-binding protein and suggesting that in trans stimulation of partition ATPases by R-fingers could be widespread. There is however a distinction between the SopB and ParG R-finger mechanisms. The nearby Arg-42 of SopB is also involved in ATPase activation since mutation to Ala abolishes it. Mutation to Lys however does not (Fig. 4A). This puts SopB in the category of certain GTPase activating proteins (GAP) that carry a secondary positively charged residue which helps orient the R-finger without contacting the active site (38). ParG does not carry such a residue.

The second aspect is the sopC stimulatory effect that makes SopB a stronger activator. Our data strongly suggest that a conformational change specifically occurs in SopB dimers upon centromere binding, and alanine 183 could be a critical residue of the hth motif involved in sensing sopC. Mutation of Ala-183 renders SopB hyperactive in SopA ATPase activation (Fig. 4A). We speculate that upon sopC-specific binding, a signal transmitted to the Nt domain alters SopB conformation, allowing optimal positioning of the Arg-36 R-finger in the SopA ATP pocket and its stabilization by Arg-42. Such a conformational change would allow the discrimination between SopB binding to nonspecific and centromeric DNA.

Since the early days of bacterial partition studies, it has been suggested that “host factors” are required for this process. However, extensive searches by several laboratories using genetical and biochemical approaches have failed to reveal specific host factors directly involved in partition. SopA, like other ParA proteins, is directly involved in the positioning of the Sop partition complexes inside the cell. By controlling SopA activities such as polymerization (12) and activation of ATP hydrolysis (Refs. 24, 25 and this study), nonspecific DNA appears to play a major role in the partition process. We propose that nonspecific DNA, represented by bacterial nucleoids, is the major host factor involved in partition.

Acknowledgments—We thank J. Rech and J. P. Castaing for technical assistance, O. Saurel for RMN determination of SopB-N32 concentration, and F. Cornet for helpful comments on the manuscript.

REFERENCES

1. Ebersbach, G., and Gerdes, K. (2005) Annu. Rev. Genet. 39, 453–479
2. Hayakawa, Y., Murotsu, T., and Matsubara, K. (1985) J. Bacteriol. 163, 349–354
3. Funnell, B. E., and Gagnier, L. (1993) J. Biol. Chem. 268, 3616–3624
4. Bouet, J. Y., Surtees, J. A., and Funnell, B. E. (2000) J. Biol. Chem. 275, 8213–8219
5. Schumacher, M. A., and Funnell, B. E. (2005) Nature 438, 516–519
6. Bouet, J. Y., and Lane, D. (2009) J. Biol. Chem. 284, 165–173
7. Gerdes, K., Møller-Jensen, J., and Bugge, Jensen, R. (2000) Mol. Microbiol. 37, 455–466
8. van den Ent, F., Møller-Jensen, I., Amos, L. A., Gerdes, K., and Löwe, J. (2002) EMBO J. 21, 6935–6943
9. Garner, E. C., Campbell, C. S., and Mullins, R. D. (2004) Science 306, 1021–1025
10. Garner, E. C., Campbell, C. S., Weibel, D. B., and Mullins, R. D. (2007) Science 315, 1270–1274
11. Bouet, J. Y., and Funnell, B. E. (1999) EMBO J. 18, 1415–1424
12. Bouet, J. Y., Ah-Seng, Y., Benmerad, N., and Lane, D. (2007) Mol. Microbiol. 63, 468–481
13. Davey, M. J., and Funnell, B. E. (1997) J. Biol. Chem. 272, 15286–15292
14. Libante, V., Thion, L., and Lane, D. (2001) J. Mol. Biol. 314, 387–399
15. Mori, H., Mori, Y., Ichinose, C., Niki, H., Ogura, T., Kato, A., and Hiraga, J. C. (2008) J. Biol. Chem. 283, 1453–1464
16. Friedman, S. A., and Austin, S. J. (1988) Plasmid 19, 103–112
17. Barilla, D., Rosenberg, M. F., Nobbmann, U., and Hayes, F. (2005) EMBO J. 24, 1453–1464
18. Ebersbach, G., Ringgaard, S., Møller-Jensen, J., Wang, Q., Sherratt, D. J., and Gerdes, K. (2006) Mol. Microbiol. 61, 1428–1442
19. Pratto, F., Cieck, A., Weihofen, W. A., Lurz, R., Saenger, W., and Alonso, J. C. (2008) Nucleic Acids Res. 36, 3676–3689
20. Marston, A. L., and Errington, J. (1999) Mol. Cell. 4, 673–682
21. Ebersbach, G., and Gerdes, K. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 15078–15083
22. Lim, G. E., Derman, A. I., and Pogliano, J. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 17658–17663
23. Hatano, T., Yamaichi, Y., and Niki, H. (2007) Mol. Microbiol. 64, 1198–1213
24. Davis, M. A., Martin, K. A., and Austin, S. J. (1992) Mol. Microbiol. 6, 1141–1147
25. Watanabe, E., Wachi, M., Yamasaki, M., and Nagai, K. (1992) Mol. Gen. Genet. 234, 346–352
26. Leonard, T. A., Butler, P. J., and Löwe, J. (2005) EMBO J. 24, 270–282
27. Hester, C. M., and Lutkenhaus, J. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 20326–20331
28. Castaing, J. P., Bouet, J. Y., and Lane, D. (2008) Mol. Microbiol. 70, 1000–1011
29. Bouet, J. Y., Rech, J., Egloff, S., Biek, D. P., and Lane, D. (2005) Mol. Microbiol. 55, 511–525
30. Bouet, J. Y., Bouvier, M., and Lane, D. (2006) Mol. Microbiol. 62, 1447–1459
31. Scheffzek, K., Ahmadian, M. R., and Wittinghofer, A. (1998) Trends Biochem. Sci. 23, 257–262
32. Ravin, N. V., Rech, J., and Lane, D. (2003) J. Mol. Biol. 329, 875–889
33. Rosinski, J. A., and Atchley, W. R. (1999) J. Mol. Evol. 49, 301–309
34. Jensen, R. B., and Gerdes, K. (1997) J. Mol. Biol. 269, 505–513
35. Yates, P., Lane, D., and Biek, D. P. (1999) J. Mol. Biol. 290, 627–638
36. McIntosh, J. R., Grishchuk, E. L., Morpew, M. K., Efremov, A. K., Zhudenkov, K., Volkov, V. A., Cheeseman, I. M., Desai, A., Mastronarde, D. N., and Ataullakhanov, F. I. (2008) Cell 135, 322–333
37. Barilla, D., Carmelo, E., and Hayes, F. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 1811–1816
38. Ahmadian, M. R., Stege, P., Scheffzek, K., and Wittinghofer, A. (1997) Nat. Struct. Biol. 4, 686–689