Stoichiometry of DnaA and DnaB Protein in Initiation at the Escherichia coli Chromosomal Origin*

Received for publication, August 3, 2001, and in revised form, September 6, 2001
Published, JBC Papers in Press, September 10, 2001, DOI 10.1074/jbc.M107463200

Kevin M. Carr and Jon M. Kaguni‡
From the Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319

Initiation of DNA replication at the Escherichia coli chromosomal origin, oriC, occurs through an ordered series of events that depend first on the binding of DnaA protein, the replication initiator, to DnaA box sequences within oriC followed by unwinding of an AT-rich region near the left border. The prepriming complex then forms, involving the binding of DnaB helicase at oriC so that it is properly positioned at each replication fork. We assembled and isolated the prepriming complexes on an oriC plasmid, then determined the stoichiometries of proteins in these complexes by quantitative immunoblot analysis. DnaA protein alone binds to oriC with a stoichiometry of 4–5 monomers per oriC DNA. In the prepriming complex, the stoichiometries are 10 DnaA monomers and 2 DnaB hexamers per oriC plasmid. That only two DnaB hexamers are bound, one for each replication fork, suggests that the binding of additional molecules of DnaA in forming the prepriming complex restricts the loading of additional DnaB hexamers that can bind at oriC.

Initiation of Escherichia coli chromosomal DNA replication involves the stepwise assembly of proteins at the chromosomal origin, oriC (reviewed in Refs. 1 and 2). This DNA locus is first recognized by the binding of DnaA protein to individual DnaA boxes in oriC in an ordered manner (3). Unwinding of an AT-rich region near the left border of oriC then occurs and requires the ATP-bound form of DnaA protein (4, 5). HU or IHF assist at this step to form an intermediate termed the open complex (6). The binding of ATP to DnaC, a prerequisite, forms the DnaB-DnaC complex (7, 8), which is then recruited to oriC to form the prepriming complex (9). At this step, DnaB interacts with two functional domains of DnaA protein; the stabilization of the prepriming complex also may involve a cryptic single-stranded DNA binding activity of DnaC (10–12). Upon the release of DnaC from the prepriming complex, DnaB is then free to act as a helicase to unwind further the parental DNA template. Its distributive interaction with primase leads to the priming of both leading and lagging strands that are then concurrently extended by each catalytic unit of the dimeric DNA polymerase III holoenzyme in the enzymatic machinery at each replication fork (Ref. 13 and references therein).

The E. coli replication origin is a locus at which DNA replication is controlled. This site also serves as the assembly site for the enzymatic machinery destined to function at each of the two replication forks that move in opposite directions on the bacterial chromosome. The work described below addresses two questions. First, what is the molecular composition of complexes formed at oriC in assembling the replication fork machinery? Second, because DnaB is the replicative helicase that drives replication fork movement, is the entry of DnaB at oriC somehow modulated so that one and only one helicase molecule is bound per replication fork, or can multiple DnaB molecules bind? The stoichiometries of DnaA and DnaB protein in the prepriming complex assembled at oriC were determined under several experimental conditions. The important observation is that the number of DnaB molecules in the prepriming complex is restricted, suggesting a mechanism to control this event.

EXPERIMENTAL PROCEDURES

Prepriming Complex Formation—Plasmid DNAs and purified proteins have been described previously (10, 14). To separate the steps of assembly of the prepriming complex from downstream events of priming and DNA replication, staged incubations were performed (15). The first stage (15-μl reaction volume assembled on ice) contained 200 ng of M13oriC2LB5 supercoiled DNA, 0.37 μg of SSB, 9 ng of HU, 90 ng of DnaA, 100 ng of DnaB, and 56 ng of DnaC protein in Buffer I (40 mM Tricine-KOH, pH 8.25, 17% (v/v) glycerol, 0.005% (v/v) Triton X-100, 0.3 mM EDTA, 0.5 mM magnesium acetate, 7 mM dithiothreitol, 0.4 mg/ml acetylated bovine serum albumin (Sigma), and 5 mM ATP) and was incubated at 37 °C for 10 min. In the indicated experiments the concentrations of ATP and magnesium acetate in Buffer I were varied. Reactions were returned to ice, then components (in a 10-μl volume) for priming and DNA replication were added so that their final concentrations or amounts (in a 25-μl reaction volume) were 0.1 mM [3H]dNTPs, 0.5 mM rCTP, rGTP, and rUTP, 8 mM creatine phosphate, 0.1 μg of creatine kinase, 0.5 μg of the A subunit of DNA gyrase, 2 μg of the B subunit of DNA gyrase, 22 ng of primase, and 25 ng of DNA polymerase III holoenzyme. The concentrations of magnesium acetate and ATP were adjusted to 10 and 3 mM, respectively, for the second stage of incubation (24 °C for 30 min). The extent of DNA synthesis was measured as trichloroacetic acid-insoluble radioactivity by liquid scintillation spectrometry.

Form I* Assay—To measure the formation of a highly underwound DNA called Form I* (16), reactions (15 μl) to assemble the prepriming complex were performed as described above with ATP at 5 mM and magnesium acetate at 0.5 mM. After incubation at 37 °C for 10 min, the reactions were returned to ice, and magnesium acetate was adjusted to 10 mM. Creatine phosphate, 8 mM; creatine kinase, 0.1 μg; A subunit of DNA gyrase, 0.5 μg; and B subunit of DNA gyrase, 2 μg; were added followed by incubation at 24 °C for 10 min. The reactions were stopped by addition of EDTA and SDS to 10 mM and 0.5% (w/v), respectively, and incubation at 65 °C for 5 min. The samples were then electrophoresed on a 0.7% agarose gel in 90 mM Tris borate, 1 mM EDTA overnight at 30 V, stained with ethidium bromide and photographed, and band intensities were determined with a Kodak EDAS 120 system.

Isolation of oriC Prepriming Complexes—Reactions of prepriming complexes (150 μl) (9, 10) contained 2 μg of either M13oriC2LB5 or M13E101 supercoiled DNA, as indicated, 3.7 μg of

* This research was supported by Grant GM33992 from the National Institutes of Health and by the Michigan Agricultural Experiment Station. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed. Tel.: 517-353-6721; Fax: 517-353-9334; E-mail: kaguni@msu.edu.
1 The abbreviations used are: oriC, chromosomal origin; SSB, single-stranded DNA-binding protein; Tricine, N-(2-hydroxyethyl)-1,1-bis-(hydroxyethyl)methylglycine; IHF, integration host factor.
RESULTS

Conditions Optimal for Prepriming Complex Formation—At oriC, assembly of the prepriming complex requires DnaA, DnaB, and DnaC and is stimulated by HU (or IHF) protein (9). The formation of this intermediate on a supercoiled DNA bearing oriC can be demonstrated by several methods. One involves its isolation by gel filtration to separate the prepriming complex from proteins not bound to the DNA, followed by measurement of its replication activity by the addition of replication proteins (SSB, primase, DNA polymerase III holoenzyme, and DNA gyrase) that act at subsequent stages of the replication process (14, 15). Alternatively, this complex can be demonstrated without its isolation in staged incubations involving its assembly in the first stage, and DNA replication in the second (15). The formation of a highly underwound oriC plasmid is a third method (16). In this assay, DnaA bound to oriC induces single-stranded character to a region of DNA near the left oriC border. The helicase activity of DnaB enlarges this region, and the compensating positive superhelicity contained in the duplex portion of the DNA can be relieved by the action of DNA gyrase. The highly negatively supercoiled DNA termed F1* is the product of this assay. Finally, prepriming complex formation can be shown by the specific retention of DnaA and DnaB (and also DnaC if the prepriming complex is formed with ATPγS instead of ATP) at oriC by immunoblot analysis of isolated complexes (10) or by electron microscopy (9, 19).

In a study by Sekimizu et al. (15), the reaction conditions optimal for forming the prepriming complex were examined. ATP and Mg2+ at 5 mM and 0.3 mM, respectively, stabilized the complex whereas a higher concentration of Mg2+ was suboptimal. To confirm the importance of these reaction conditions, experiments were done to measure the assembly of the prepriming complex in staged incubations. In the first stage, DnaA, DnaB, DnaC, and HU were incubated with the oriC plasmid. ATP and Mg2+ concentrations were varied in the first stage of incubation. In the second stage, their concentrations were adjusted to 3 mM ATP and 10 mM Mg2+, and primase, DNA polymerase III holoenzyme, SSB, and DNA gyrase were added to measure DNA synthesis. The results of Fig. 1 show that among the concentrations of ATP and Mg2+ tested, formation of the prepriming complex occurs best at low Mg2+ (0.5 mM) and at 5 mM ATP. Under these conditions, DNA replication is absolutely dependent on DnaA protein and a supercoiled template carrying oriC. (Fig. 2). Furthermore, this DNA replication activity correlated with a more direct assay of prepriming complex formation as measured by F1* formation (Fig. 3). By agarose gel electrophoresis, this highly underwound DNA migrates more rapidly than the supercoiled oriC plasmid (Form I) isolated from E. coli. Its formation requires assembly of the prepriming complex in which DnaA protein at oriC first unwinds a region of duplex DNA within oriC followed by entry of DnaB from the DnaB-DnaC complex. Once DnaB has bound to the DNA template, its activity as a DNA helicase enlarges the opened region and induces positive superhelicity elsewhere on the duplex portion of the DNA. Addition of DNA gyrase relieves the positive superhelicity; further combined activities of DnaB and gyrase produce Form I* that is highly negatively supercoiled.

Assembly of the Prepriming Complex Requires oriC—In our experience, DnaA protein has a natural tendency to self-aggregate, and to bind to DNA nonspecifically when assay conditions are not appropriate (3). Assays like those above that measure the activity of the prepriming complex fail to detect the formation of inactive complexes resulting from nonspecific binding of DnaA and other prepriming proteins to DNA. To provide more support for the importance of reaction conditions in assembly of the prepriming complex at oriC, experiments were done to measure the binding of prepriming proteins to either the oriC DNA or the vector as a negative control when incubated at various concentrations of ATP and Mg2+. As will be shown below, ATP at 5 mM and Mg2+ at 0.5 mM promote the specific assembly of the prepriming complex at oriC whereas omission of Mg2+ or low ATP (0.1 mM) results in nonspecific binding of DnaA and DnaB to the vector DNA. After incubation, proteins bound to each respective DNA were separated from unbound protein by gel filtration chromatography. Fractions from the column were analyzed by agarose gel electrophoresis to detect the DNA (Fig. 4, A and D) and by immunoblotting with antibodies specific for each prepriming protein (Fig. 4, C–F). At 0.1 mM ATP, a level sufficient to saturate the ATP binding site of
DnaA protein but in the absence of Mg\(^{2+}\), the amount of DnaA bound to the vector plasmid was comparable with that bound to the oriC DNA (Fig. 4, C and D), indicating nonspecific binding. Whereas DnaB bound preferentially to the oriC plasmid, the level of DnaB coeluting with the vector DNA (Fig. 4F) may be because of its ability to bind to DnaA (10, 11) that, under these conditions, is binding nonspecifically to this DNA. In this experiment, DnaC was not detected because of an insufficient amount of affinity-purified anti-DnaC antibody. However, we did not expect DnaC to be retained in the prepriming complex because ATP supports the release of DnaC from DnaB once the helicase is bound to DNA (11, 20). The dissociation of DnaC from DnaB is dependent on ATP hydrolysis because the replacement of ATP with a nonhydrolyzable ATP analogue (ATP-γS) results in the retention of DnaC to DnaB (20).²

Prepriming complexes assembled under other conditions were also analyzed by immunoblotting. At a higher level of ATP (5 mM compared with 0.1 mM ATP in Fig. 4) but without Mg\(^{2+}\), DnaA was bound to the vector DNA at a comparable level as in Fig. 4E (data not shown). This observation suggests that DnaA probably binds to ATP as a Mg-ATP chelate. Lowering the ratio of DnaA protein to DNA prior to gel filtration chromatography did not reduce this background of nonspecific binding of DnaA to DNA. Elevating the Mg\(^{2+}\) concentration to 10 mM in the presence of 0.1 mM ATP, specific binding of DnaA to the oriC plasmid was observed with no detectable binding of DnaA protein to the vector. However, the level of DnaA bound under this condition is suboptimal for DNA replication activity of the prepriming complex (Fig. 1).

Stoichiometry of DnaA and DnaB at oriC—At 5 mM ATP and 0.5 mM Mg\(^{2+}\), conditions optimal for prepriming complex formation (Figs. 1–3), prepriming complexes were isolated by gel filtration chromatography and analyzed to determine the stoichiometry of specific proteins to oriC DNA by quantitative Western blotting (see Fig. 5 for examples). The amount of DNA was determined by quantitation of ethidium bromide-stained agarose gels relative to known amounts of DNA that were co-electrophoresed and used to prepare a standard curve. Under these conditions, the binding of DnaA to the oriC plasmid was specific. In contrast, if for example ATP was omitted from the buffer for gel filtration chromatography, the ratio of DnaA per oriC was 27 monomers per DNA (Table I, line 4). As ATP minimizes nonspecific aggregation (3), the ratio of DnaA protein per oriC DNA measured here may not be physiologically relevant. Recall also that nonspecific binding of DnaA and DnaB to the vector DNA occurs when Mg\(^{2+}\) was omitted from the column buffer (Fig. 4). These observations emphasize the importance of reaction conditions when measuring the binding of DnaA to DnaA box sequences to derive values on stoichiometry; DnaA binds DNA nonspecifically in the absence of ATP or Mg\(^{2+}\).

Quantitative Western blotting was used to determine the stoichiometry of prepriming proteins specifically bound to oriC. A representative experiment is shown in Fig. 5. Several identical experiments were performed to obtain statistical values which show a stoichiometry of 10 DnaA monomers and 2 DnaB hexamers per oriC plasmid. Because five DnaA boxes are present within oriC (21, 22), this value corresponds to two DnaA monomers per DnaA box. Replication forks that progress from oriC move bidirectionally on the circular bacterial chromosome. The two DnaB hexamers correspond to one helicase molecule per replication fork. Because the prepriming complex was assembled with ATP, DnaC that was originally in the DnaB-DnaC complex was not retained in the prepriming complex.

² J. Kaguni, unpublished results.
ATP hydrolysis by DnaB is presumed to move the helicase from its original site of entry.

Interestingly, in the absence of DnaB and DnaC protein, the stoichiometry of DnaA bound to oriC was 4.2 ± 1.1 monomers. Given that oriC bears five DnaA boxes (21, 22), it is attractive to interpret this stoichiometry to mean that each of the five

(data not shown). ATP hydrolysis by DnaB is presumed to move the helicase from its original site of entry.

Interestingly, in the absence of DnaB and DnaC protein, the stoichiometry of DnaA bound to oriC was 4.2 ± 1.1 monomers. Given that oriC bears five DnaA boxes (21, 22), it is attractive to interpret this stoichiometry to mean that each of the five

(data not shown). ATP hydrolysis by DnaB is presumed to move the helicase from its original site of entry.

Interestingly, in the absence of DnaB and DnaC protein, the stoichiometry of DnaA bound to oriC was 4.2 ± 1.1 monomers. Given that oriC bears five DnaA boxes (21, 22), it is attractive to interpret this stoichiometry to mean that each of the five
DnaA boxes is bound by a single DnaA monomer whereas when DnaB has become stably bound to oriC, two monomers are bound per DnaA box. Apparently, the binding of additional DnaA molecules at oriC is coordinated with formation of the prepriming complex.

DISCUSSION

Stoichiometry of Prepriming Proteins at oriC—DNA replication is initiated at oriC through a stepwise process involving the assembly of an intermediate termed the prepriming complex (reviewed in Refs. 1 and 2). Its formation requires the binding of DnaA protein to the DnaA boxes within oriC followed by the local unwinding of an AT-rich region near the left oriC boundary. The recruitment of DnaB in the DnaB-DnaC complex then follows concomitantly with assembly of DnaA dimers at each DnaA box. The hydrolysis of ATP bound by DnaC in the DnaB-DnaC complex releases DnaC. Bound to each parental DNA template, DnaB then moves in the 5’ to 3’ direction and interacts periodically with primase, priming both leading- and lagging-strand synthesis.

Stoichiometry of DnaA and DnaB at oriC

The results from identical sets of experiments were averaged to calculate the standard deviation for the stoichiometry of DnaA and DnaB protein. The values under the column labeled N represent the number of times the experiment was performed. The range in the ratios of DnaA and DnaB per oriC plasmid is also indicated.

| Conditions                           | Protein | Monomers per oriC | N | Range      |
|-------------------------------------|---------|-------------------|---|------------|
| Complete prepriming mix             | DnaA    | 9.9 ± 1.4         | 5 | 8.5–12.0   |
|                                     | DnaB    | 12.0 ± 0.4        | 3 | 11.6–12.4  |
| No DnaB-DnaC                        | DnaA    | 4.2 ± 1.1         | 4 | 3.3–5.8    |
| No ATP in column buffer             | DnaA    | 27 ± 2.3          | 3 | 25–29      |
| M13ΔE101 in place of M13oriC2LB5    | DnaA    | ≤0.5              |   |            |

Fig. 6. Initial steps at oriC. In the first step, DnaA at oriC binds to the respective DnaA boxes sequentially, as indicated by the superimposed numbers. Complexed to ATP, DnaA then opens an AT-rich region that contains 13-mer sequences designated L, M, and R near the left oriC boundary. The recruitment of DnaB in the DnaB-DnaC complex then follows concomitantly with assembly of DnaA dimers at each DnaA box. The hydrolysis of ATP bound by DnaC in the DnaB-DnaC complex releases DnaC. Bound to each parental DNA template, DnaB then moves in the 5’ to 3’ direction and interacts periodically with primase, priming both leading- and lagging-strand synthesis.

DnaA boxes is bound by a single DnaA monomer whereas when DnaB has become stably bound to oriC, two monomers are bound per DnaA box. Apparently, the binding of additional DnaA molecules at oriC is coordinated with formation of the prepriming complex.

Stoichiometry of Prepriming Proteins at oriC—DNA replication is initiated at oriC through a stepwise process involving the assembly of an intermediate termed the prepriming complex (reviewed in Refs. 1 and 2). Its formation requires the binding of DnaA protein to the DnaA boxes within oriC followed by the local unwinding of an AT-rich region near the left oriC boundary. The recruitment of DnaB in the DnaB-DnaC complex then binds, which must then lead to the proper positioning of two helicase molecules for bidirectional replication fork movement. The study described here determined the stoichiometry of prepriming proteins bound to oriC and is the first quantitative determination of the number of DnaA monomers alone and in the prepriming complex assembled on a supercoiled oriC plasmid. The results from immunoblotting are very reproducible among replicate experiments. Considering the error in measurements, DnaA alone binds with a stoichiometry of four to five DnaA monomers per oriC. This value coincides with the five DnaA boxes in oriC. The conclusion that a single monomer is bound to each box is based on this observation and others. Gel mobility shift experiments with DnaA bound to oriC reveals six shifted complexes (named Complex I through VI) (3). In situ footprinting showed that Complexes I through IV are formed by the binding of DnaA to DnaA boxes R4, R1, R2, then R3, respectively. Although we were unable to detect if DnaA was bound to DnaA box R5, complex V (or VI) may be formed by this binding event. We suggest that complex VI (or conversely complex V if complex VI arises by the binding of DnaA to the R5 box) then forms by a conformational change in the oriC fragment induced by the interaction among bound DnaA monomers. Finally, gel mobility shift experiments with radiolabeled DnaA protein and an oligonucleotide carrying DnaA box R4 revealed a stoichiometry of 0.8/H11006 DnaA monomer per DNA (23). In the dnaA promoter region, the stoichiometry was one monomer per DnaA box as determined by surface plasmon resonance (24). These observations are consistent with our findings.

The increase in stoichiometry to 10 DnaA monomers per oriC plasmid on inclusion of the DnaB-DnaC complex is the first evidence that the prepriming complex results in the recruitment of additional DnaA molecules upon DnaB binding. Because an N-terminal domain of DnaA is involved in oligomer-
ization and in retention of DnaB in the prepriming complex (11, 25), this domain is apparently involved both in the uptake of additional DnaA monomers and DnaB in the prepriming complex. The stoichiometry of two and only two DnaB hexamers suggests that a mechanism to control this process.

DnaA bound to oriC alone and in the prepriming complex has been examined by electron microscopy (9, 19). The samples were first fixed with glutaraldehyde then shadowed with tungsten vapor to enhance contrast. Although the method is inherently imprecise, the estimate of 20–40 monomers of DnaA bound to oriC is in reasonable agreement with our findings. The larger size of the prepriming complex compared with the DnaA-oriC complex when viewed by electron microscopy is also consistent with our immunoblotting results.

**Conditions for Formation of the Prepriming Complex—ATP and Mg^{2+} concentrations are important in assembling the prepriming complex (Fig. 1). In the absence of Mg^{2+} or ATP, DnaA nonspecifically binds to the vector DNA (Table I, line 4; Fig. 4). DnaA is most likely in the form of a nonspecific aggregate because in the absence of either cofactor, DnaA binds to any DNA fragment. The large size of this protein-DNA complex is indicated by its retention near the sample well in gel mobility shift assays (3). In the absence of Mg^{2+} in the reaction buffer, DnaB also associates with the vector DNA when it should not be (Fig. 4). By comparison under conditions appropriate for prepriming complex formation, DNA replication is specific for supercoiled DNA carrying oriC (Fig. 3).

A recent study described steps in assembling the replication fork machinery at oriC (26). Primers in the vicinity of oriC served to recruit DNA polymerase III holoenzyme to form a replicative complex, requiring the prior assembly of the prepriming complex. In the cited report, DnaB was modified to carry a protein kinase site to label it radioactively so that its ratio to the ^3H-labeled DNA template could be calculated. Despite the isolation of the replicative complex in buffer lacking ATP, the stoichiometry of DnaB bound to the forks was near two, in keeping with the bidirectional mode of replication and the stoichiometry of two hexamers that we determined here. The modified DnaB retains full activity as a single-stranded DNA-dependent ATPase (27). It was also active in stimulating primase in general priming, but had 80% of wild type activity in oriC plasmid replication. The reduced activity suggests the possibility that the protein kinase site may perturb the activity in primase in general priming, but had 80% of wild type activity in oriC plasmid replication. The reduced activity suggests the possibility that the protein kinase site may perturb the activity.

**Positioning of DnaB Helicase at oriC—DnaB binds poorly if at all to duplex DNA or to single-stranded DNA covered by SSB (28). In physiologically relevant systems, an active process is involved in loading the helicase onto single-stranded DNA. In the single-stranded DNA sequence, PriA with other primosomal proteins forms a complex that is then recognized by the DnaB-DnaC complex (29). In recombination-dependent DNA replication, PriA is also thought to load DnaB at D loop structures (reviewed in Ref. 30). In the prepriming complex, DnaC must be in a complex with DnaB for helicase to be loaded. The involvement of DnaC in helicase loading may involve its ability to bind to single-stranded DNA (12).

The binding site size of DnaB for single-stranded DNA is a polynucleotide of 20 residues. DnaA unwinds an AT-rich region near the left border of oriC, and KminO4 probing indicates that 26 base pairs in this region becomes single stranded (31). Based on these observations, an attractive model is that the unwound region is the site where DnaB initially binds to the DNA. Recent footprinting studies of DnaB bound at oriC support this model (26).

**The finding that only two DnaB hexamers are bound in the prepriming complex suggests that the limited amount of available single-stranded DNA controls the number of DnaB hexamers that can bind. This model seems very unlikely for two reasons. First, it is estimated that a 6.6-kilobase supercoiled oriC plasmid used in our experiments has about 6% single-stranded character, based on electron microscopy of unwound structures induced by DnaB helicase activity in the absence of DNA gyrase (32). This estimate is supported by the limited extent of DNA synthesis that is localized to the oriC region when DNA replication occurs on a supercoiled template with the necessary proteins but in the absence of DNA gyrase (15). Second, in a replication system in which DnaB is delivered onto a single-stranded DNA covered by SSB by the binding of DnaA to a DnaA box hairpin, only one DnaB hexamer is bound. A mechanism must act to limit the number of DnaB hexamers to one per single strand template. The 2-fold increase in the number of DnaA molecules bound to oriC upon binding of DnaB suggests a mechanism to modulate DnaB binding.

**A Model for Assembly of the Prepriming Complex—In this model (Fig. 6), DnaA monomers bound to each DnaB box in oriC creates a structure recognized by the DnaB-DnaC complex. This then promotes the binding of additional DnaA monomers that not only stabilize the binding of DnaB, but also blocks the subsequent binding of more DnaB hexamers. Because of the 2-fold increase in stoichiometry of DnaA at this step, and the report that Streptomyces DnaA binds to a single DnaA box as a dimer (33), it is attractive to think that each DnaA box is bound by a dimer of DnaA. Once DnaB is bound to the template, DnaC is released. Driven by ATP hydrolysis, a single DnaB hexamer bound to each template strand can then move in the 5' to 3' direction from its original site of binding. It can interact with primase to prime leading-strand synthesis by the first primer and lagging-strand synthesis by primers made subsequently (34, 35).

**REFERENCES**

1. Messer, W., and Weigel, C. (1996) in Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 2, 2nd Ed., pp. 1579–1601, American Society for Microbiology, Washington, D. C.

2. Kaguni, J. M. (1997) Mol. Cells 7, 145–157

3. Margulies, C., and Kaguni, J. M. (1996) J. Biol. Chem. 271, 17035–17040

4. Bramhili, D., and Kornberg, A. (1988) Cell 52, 743–755

5. Sekimizu, K., Bramhili, D., and Kornberg, A. (1987) Cell 50, 259–265

6. Hwang, D. S., and Kornberg, A. (1992) J. Biol. Chem. 267, 23083–23086

7. Wahle, E., Lasken, B. S., and Kornberg, A. (1989) J. Biol. Chem. 264, 2463–2468

8. Galletto, R., Rajendran, S., and Bujalowski, W. (2000) Biochemistry 39, 12859–12866

9. Funnell, B. E., Baker, T. A., and Kornberg, A. (1987) J. Biol. Chem. 262, 10327–10334

10. Marszalek, J., and Kaguni, J. M. (1994) J. Biol. Chem. 269, 4883–4890

11. Sutton, M. D., Carr, K. M., Vicente, M., and Kaguni, J. M. (1998) J. Biol. Chem. 273, 34255–34262

12. Learn, B. A., Um, S. J., Huang, L., and McMacken, R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1154–1159

13. Glover, B. B., and McHenry, C. S. (2001) J. Biol. Chem. 276, 533

14. Marszalek, J., and Kaguni, J. M. (1992) J. Biol. Chem. 267, 19343–19349

15. Sekimizu, K., Bramhili, D., and Kornberg, A. (1988) J. Biol. Chem. 263, 7124–7130

16. Baker, T. A., Sekimizu, K., Funnell, B. E., and Kornberg, A. (1986) Cell 45, 53–64

17. Marszalek, J., Zhang, W., Hupp, T. B., Margulies, C., Carr, K. M., Cherry, S., and Kaguni, J. M. (1996) J. Biol. Chem. 271, 18535–18542

18. van der Ende, A., Baker, T. A., Ogawa, T., and Kornberg, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3954–3958

19. Crooke, E., Thureau, H., Hwang, D. S., Griffith, J., and Kornberg, A. (1993) J. Mol. Biol. 233, 16–24

20. Wahle, E., Lasken, R. S., and Kornberg, A. (1989) J. Biol. Chem. 264, 2449–2475

21. Fuller, R. S., Funnell, B. E., and Kornberg, A. (1984) Cell 38, 889–900

22. Matsui, M., Oka, A., Takekami, M., Yasuda, S., and Hirota, Y. (1985) J. Mol. Biol. 184, 529–533

23. Schaper, S., and Messer, W. (1995) J. Biol. Chem. 270, 17622–17626

K. M. Carr and J. M. Kaguni, manuscript in preparation.
Stoichiometry of DnaA and DnaB at oriC

24. Speck, C., Weigel, C., and Messer, W. (1999) EMBO J. 18, 6169–6176
25. Sutton, M. D., and Kaguni, J. M. (1997) J. Mol. Biol. 274, 546–561
26. Fang, L., Davey, M. J., and O’Donnell, M. (1999) Mol Cell 4, 541–553
27. Yushakov, A., Turner, J., and O’Donnell, M. (1996) Cell 86, 877–886
28. Arai, K., and Kornberg, A. (1981) J. Biol. Chem. 256, 5253–5259
29. Marians, K. J. (1992) Annu. Rev. Biochem. 673–719
30. Cox, M. M., Goodman, M. F., Kreuzer, K. N., Sherratt, D. J., Sandler, S. J., and Marians, K. J. (2000) Nature 404, 37–41
31. Gille, H., and Messer, W. (1991) EMBO J. 10, 1579–1584
32. Baker, T. A., Funnell, B. E., and Kornberg, A. (1987) J. Biol. Chem. 262, 6877–6885
33. Majka, J., Zakrzewska-Czerwinska, J., and Messer, W. (2001) J. Biol. Chem. 276, 6243–6252
34. Wu, C. A., Zechner, E. L., Reems, J. A., McHenry, C. S., and Marians, K. J. (1992) J. Biol. Chem. 267, 4074–4083
35. Tougu, K., Peng, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 4675–4682
Stoichiometry of DnaA and DnaB Protein in Initiation at the Escherichia coli Chromosomal Origin

Kevin M. Carr and Jon M. Kaguni

J. Biol. Chem. 2001, 276:44919-44925.
doi: 10.1074/jbc.M107463200 originally published online September 10, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107463200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 19 of which can be accessed free at http://www.jbc.org/content/276/48/44919.full.html#ref-list-1