An Essential Tryptophan of *Escherichia coli* DnaA Protein Functions in Oligomerization at the *E. coli* Replication Origin*

Received for publication, April 4, 2005  
Published, JBC Papers in Press, May 5, 2005, DOI 10.1074/jbc.M503684200

Magdalena M. Felczak, Lyle A. Simmons‡, and Jon M. Kaguni§

From the Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319

In the initiation of bacterial DNA replication, DnaA protein recruits DnaB helicase to the chromosomal origin, oriC, leading to the assembly of the replication fork machinery at this site. Because a region near the N terminus of DnaA is required for self-oligomerization and the loading of DnaB helicase at oriC, we asked if these functions are separable or interdependent by substituting many conserved amino acids in this region with alanine to identify essential residues. We show that alanine substitutions of leucine 3, phenylalanine 46, and leucine 62 do not affect DnaA function in initiation. In contrast, we find on characterization of a mutant DnaA that tryptophan 6 is essential for DnaA function because its substitution by alanine abrogates self-oligomerization, resulting in the failure to load DnaB at oriC. These results indicate that DnaA bound to oriC forms a specific oligomeric structure, which is required to load DnaB helicase.

Chromosomal DNA replication origins function as sites where enzymes assemble to form the replication fork machinery. In *Escherichia coli*, DnaA directs the assembly process by first recognizing and binding to DnaA box motifs as well as to I sites within the chromosomal origin, oriC (1, 2). Complexed to ATP, DnaA next unwinds a region within oriC and then recruits the replicative helicase, DnaB, to form the prepriming complex (3, 4). After DnaB translocates to the apex of each replication fork, the helicase both unwinds the parental DNA and interacts transiently with primase (5, 6). Primers synthesized by primase are then extended by DNA polymerase III holoenzyme under a bidirectional mode of DNA replication. In *S. cerevisiae*, the ORC proteins (Orc1–6) perform an analogous function, recognizing the replication origin to recruit Mcm2–7, the eukaryotic counterpart to *E. coli* DnaB (reviewed in Ref. 7). The remarkable similarity of the three-dimensional structure of a truncated form of DnaA protein from *Aquifex aeolicus* to the corresponding region of archaeal Cdc6/Orc1 (8) and their function at initiation suggest that all organisms utilize a common biochemical pathway.

Because of the critical importance of DnaA in chromosomal replication and regulating this event, structure-function studies have been performed to understand its activities (reviewed in Refs. 9 and 10). These studies reveal that DnaA comprises several functional domains (Fig. 1A). Some of these domains have been mapped to the predicted three-dimensional structure of a portion of *E. coli* DnaA, obtained by homology modeling to the crystal structure of the corresponding C-terminal two-thirds of *A. aeolicus* DnaA (8). This structure lacks the N-terminal third of DnaA, which functions to recruit DnaB helicase to oriC (4, 11) and to oligomerize DnaA during initiation (12).

Several studies support the conclusion that the N-terminal region of DnaA is needed for self-oligomerization. In one study, mutations mapping to the N-terminal region were defective in autoregulation of dnaA expression (13). Because autoregulation appears to require DnaA oligomerized at the dnaA promoter region (14), these alleles are apparently defective in this function. In support, a separate study showed that this region (residues 2–86) could replace the dimerization domain of bacteriophage λCI repressor in transcriptional repression of the λ Fβ promoter (15). A third report identified specific amino acids in a predicted α-helix proximal to the N terminus (Fig. 1B) that functions in self-oligomerization, using a genetic assay measuring complementation between two inactive dnaA alleles (12). These findings show that self-oligomerization of DnaA at oriC is essential for initiation.

Amino acid sequence comparison of homologous DnaAs identified moderately and more highly conserved residues in the N-terminal region of DnaA (Fig. 1B), suggesting their functional importance. We undertook a mutational approach to identify amino acids that are critical for function and to ask if the functions of DnaB retention at oriC and self-oligomerization are separable. Biochemical characterization of a particular mutant DnaA carrying an alanine substitution of tryptophan at the sixth position of the primary sequence (W6A) shows that these activities are coupled. Because leucines are unusually abundant in the N-terminal 70 amino acids (Fig. 1), we also asked whether many of these leucines are functionally important. Specifically, we explored whether some of these form a variant of the leucine-rich repeat motif, because inspection did not reveal the signature motif (LXXLXLLXX(N/C)XLL, where X is any amino acid) involved in protein-protein interactions (reviewed in Ref. 16). Because of proteolysis of specific mutant DnaAs, it is not clear whether the leucines examined are essential for function.

**EXPERIMENTAL PROCEDURES**

*Replication Proteins—*Wild type DnaA protein fused at its N terminal to polyhistidine (His-Tag®, Novagen) was purified from an overproducing strain carrying pKC597 (see below) as described (17), but the step of heparin-agarose chromatography was substituted by metal chelation chromatography. Monomeric His-tagged DnaA is essentially identical to wild type DnaA in *oriC* plasmid replication in either a

---

* This work 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.

†Present address: Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, MA 02139.

‡To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824-1319. Tel.: 517-353-6721; Fax: 517-353-9334; E-mail: kaguni@msu.edu.
FIG. 1. Domains of E. coli DnaA. In A at the top, secondary structures of E. coli DnaA are derived from the predicted structure of E. coli DnaA and a homology model of DnaA (18) with the corresponding portion of A. aeolicus DnaA (8, 31). The Walker A and B boxes, Sensors I and II (Box VIII), and Box VII motifs, which are conserved among AAA+ proteins, and the portion lacking in DnaAΔ62 are shown as black bars. Functional domains involved in DnaA oligomerization (13, 42), in retention of DnaB in the oriC prepriming complex (11, 43), in interaction with DnaB (11, 43), membrane binding (residues 372–381; Ref 44), and DNA binding (29, 34) are described in the cited references. In B, the sequence of the N-terminal region of E. coli DnaA is shown, with conserved and moderately conserved amino acids (13) highlighted in red and blue, respectively.

RESULTS

Genetic Analysis of the N-terminal Region of DnaA—We investigated the function of 10 amino acid residues contained within an N-terminal region of DnaA (see Fig. 1), asking whether these residues are essential. As previous results implicate the function of this region in self-aggregation (12), we also wondered whether those leucines found to be essential form a variant of the leucine rich motif, which is involved in protein-protein interactions (16). By site-directed mutagenesis, the respective codons were altered to encode alanine, the missense mutations were confirmed by DNA sequence analysis of the dnaA gene and the upstream regulatory region, and the alleles carried in pBR322 were analyzed for their ability to maintain an oriC plasmid upon its transformation. To exclude the contribution of the chromosomal dnaA gene, the host strain (MS3988; see genotype in the legend to Fig. 2) lacked the chromosomal dnaA locus. As a control, we included the P28L allele that we characterized previously (20). Its partial activity appears to be consistent with earlier observations that P28L is active at 30 °C but less so at 42 °C. The results indicate that L3A, F46A, and L62A were comparable or nearly comparable with wild type dnaA in supporting DNA replication of the oriC plasmid, whereas L10A was about 3-fold reduced in activity. Compared with previous studies of an L10S substitution (27), which showed no effect on DnaA function in initiation, substitution of leucine 10 by alanine caused a modest reduction in activity. The remaining alleles either failed to maintain the oriC plasmid, or were greatly reduced in function as with L38A.

In interpreting the phenotypes of the alleles, we were concerned that an amino acid substitution may alter protein conformation and thus increase the susceptibility of a mutant DnaA to proteolysis. Consequently, the inactive phenotype of an allele may arise from a partially proteolyzed protein, which is non-functional, or an inadequate steady-state level for oriC plasmid maintenance. To address this concern, we performed immunoblot analysis to approximate roughly the levels of the mutant proteins relative to DnaA + and to determine whether any were proteolyzed. Compared with DnaA +, we found that L3A, F46A, and L62A were indistinguishable in size, and their steady-state levels were similar or elevated severalfold (Fig. 2). For these mutants, we conclude that the amino acid substitution does not...
Tryptophan 6 Is Required for DnaA Oligomerization

**A**

| Plasmid | Amino Acid Substitution | Steady State Level | Relative Transformation by pCM959-Cm\(^{R}\) |
|---------|-------------------------|-------------------|---------------------------------|
| pBR322  | -                       |                   | -\(^{<10^{-4}}\)                   |
| pRB100  | dnaA\(^{+}\)            |                   | 1.0                             |
| pRBL3A  | L3A                     | 1.0               | 1.2                             |
| pRBL6A  | W6A                     | 0.3               | \(^{<10^{-4}}\)                  |
| pRBB1A  | L10A                    | 0.4               | 0.3                             |
| pRBL3A  | L13A                    | not detected      | \(^{<10^{-4}}\)                  |
| pRBL17A | L17A                    | 0.5               | 0.3                             |
| pRBB2B  | P28B                    | 1.0               | 0.2 (0.1)                       |
| pRBL7A  | L38A                    | 0.65              | 0.15                            |
| pRBL40A | L40A                    | 0.9               | 0.2                             |
| pRBB46A | F46A                    | 3.2               | 0.5                             |
| pRBB59A | F59A                    | 0.5               | 0.2                             |
| pRBB62A | L62A                    | 0.9               | 0.9                             |

* Measured at 42°C

**B**

![Diagram](http://www.jbc.org/)

**FIG. 2.** Assays of plasmid maintenance coupled with immunoblot analysis identify mutant DnaAs that retain function and also proteolysed forms of other mutant DnaAs. In A, *E. coli* MS3898 (asnB32 relA1 spoT1 thi-1 ilv-192 zia::pKN500 (pKN500 = mini-R1 ΔdnaA mad-2 (F\(^{+}\)) recA1 (λimm434) (26)) carrying pBR322, pRB100, which expresses the dnaA\(^{+}\) allele from the natural dnaA promoters, or its derivatives encoding the indicated mutations was transformed by electroporation with either the oriC plasmid pCM959-Cm\(^{R}\) or pACYC184 as a control, followed by incubation of LB plates containing the appropriate antibiotics at 37 °C unless indicated. pACYC184, which is not dependent on dnaA encoding the indicated mutations was transformed by electroporation with either the DnaA or the respective mutant DnaAs was grown in LB medium supplemented with 50 μg/ml ampicillin at 37 °C unless noted otherwise. At a range of turbidity from 0.4 to 0.6 OD (595 nm), cells were collected by centrifugation, resuspended in Laemmli sample buffer, and the lysate electrophoresed under reduced conditions in 10% polyacrylamide gels containing SDS. Except for the membrane with L10A, the membrane-immobilized proteins were detected with M43 monoclonal antibody, which recognizes an epitope within residues 133–141 of DnaA protein (25). The membrane with L10A was probed with rabbit antiserum prepared against a polypeptide containing the C-terminal 89 amino acids of DnaA (data not shown). These results suggest that L13A is rapidly degraded. A portion of the remaining mutant DnaAs was partially proteolyzed, supporting the idea that these appear inactive not because the substitution affects function but because proteolyzed forms interfere with the activity of the intact protein at oriC. Because we detected similar partially proteolyzed forms with either antibody (Fig. 2; data not shown), these results suggest that proteolytic cleavage is within the N-terminal 133 residues.

**Tryptophan 6 Is Required for DnaA Function at oriC**—Because tryptophan 6 is highly conserved, we focused among the defective mutants on characterizing the mutant DnaA carrying the W6A substitution in initiation at oriC. To discern whether the inability of W6A to maintain the oriC plasmid is from the mutation itself or arises from interference by a proteolyzed form, we undertook a biochemical approach, purifying the intact protein from an overproducing strain. Interestingly, we found no evidence of proteolysis after induced expression of the mutant DnaA or at various steps of purification (see comments under “Discussion”).

We then characterized purified W6A in several biochemical assays. In measuring its activity in oriC plasmid replication, we found that W6A was almost inert (Fig. 3A).

Because the oligomerization of DnaA at oriC is required for initiation (12), W6A added to reactions containing wild type DnaA should inhibit oriC plasmid replication if the defect of a single W6A protomer inactivates the function of the DnaA oligomer. If W6A is inactive in self-oligomerization, the mutant DnaA should not inhibit DnaA\(^{+}\) activity in initiation. To distinguish between these possibilities, we added increasing amounts of the mutant DnaA to reactions containing a near optimal but subsaturating amount of DnaA\(^{+}\) protein (50 ng). At the lowest level of W6A (25 ng), the modest stimulation most likely reflects experimental variability because the level of DNA synthesis is comparable with that obtained with no additional DnaA\(^{+}\) protein (Fig. 3B). Compared with these levels of
DNA synthesis, the relative inhibition observed with W6A at higher amounts was similar to that seen with DnaA/H11001. Thus although W6A is essentially inactive by itself, it apparently does not severely interfere with initiation by wild type DnaA, suggesting that W6A may fail to oligomerize with DnaA/H11001 bound to oriC.

Interestingly, the inactivity of W6A in initiation with a supercoiled oriC plasmid contrasts with its activity with a single-stranded DNA carrying a DnaA box in a hairpin structure (Fig. 3C). With this DNA, DnaA binds to the DnaA box hairpin to recruit DnaB helicase, which in turn interacts with primase to synthesize primers for DNA replication (22, 28). These primers are extended by DNA polymerase III holoenzyme to convert the single-stranded DNA to duplex form. These results indicate that the deficiency of W6A is specific for oriC and that the substitution does not affect either helicase recruitment or sequence-specific DNA binding, the latter activity for which we confirm directly below.

W6A Is Active in Binding to the DnaA Boxes of oriC and RK2 oriV—Because the DNA binding domain of DnaA is contained in its C-terminal 94 amino acids (29), we expected that recognition of the DnaA box sequence by W6A would be unimpaired. To confirm our expectation, gel mobility shift analysis was done with a DNA fragment carrying oriC (Fig. 4A). We substantiated previous observations that wild type DnaA forms six discrete complexes, which reflect the binding of DnaA to the individual DnaA boxes within oriC and the different affinities of DnaA to these sites (30). W6A bound comparably to this DNA fragment as DnaA/H11001, and the small differences of the mutant DnaA compared with the control arise from experimental error based on a comparison of this experiment to others like it. These results indicate that the amino acid substitution does not alter sequence-specific DNA binding.

We also measured DNA binding to the RK2 plasmid replication origin (oriV) (Fig. 4B), which has four DnaA boxes organized as two sets of inverted repeats (31). A low ratio of DnaA to oriV, previous gel mobility shift analyses showed that a complex named Complex I is never seen in the absence of Complex II (24). Complex I of greater electrophoretic mobility than Complex II is retarded compared with the unbound oriV fragment. These results suggest that DnaA molecules bound to individual DnaA boxes interact to form Complex II. In support, DNA binding is sequential if the rightmost DnaA compared with the control arise from experimental error based on a comparison of this experiment to others like it. These results indicate that the amino acid substitution does not alter sequence-specific DNA binding.

Because of recent evidence that residues in an N-terminal region of DnaA function in self-oligomerization (12), and the possibility that Complex II formation at oriV may involve cooperative binding, we examined W6A in gel mobility shift assays with an oriV-containing DNA. At lower DnaA+ levels, we...
confirmed that Complex I was not detected without Complex II. W6A was essentially identical to DnaA/H11001 in binding to this DNA, indicating that the W6A substitution does not affect the binding to oriV.

**W6A Is Active in ATP Binding and ATP Hydrolysis**—High affinity ATP binding by DnaA protein is required for initiation at oriC (32). To find if the W6A substitution affected ATP binding, we performed nitrocellulose filter binding assays, which showed that the mutant DnaA bound ATP with an affinity comparable to DnaA/H11001 (KD of 0.14 and 0.1 µM, respectively; Fig. 5 A). The ratio of ATP bound per monomer was also similar (0.29 ATP/W6A; 0.24 ATP/DnaA/H11001).

DnaA is also a weak ATPase (32). ATPase assays revealed that the rate of ATP hydrolysis by W6A, as indicated by the appearance of W6A complexed to ADP, was similar to DnaA+ (Fig. 5B). After 30-min incubation, the greater amount of DnaA complexed to ADP with W6A-ADP appears to be from the increased amount of ATP bound by DnaA+ than by W6A at the onset of this experiment. Summarizing the above results, the inactivity of W6A in oriC plasmid replication is not from defects in nucleotide hydrolysis or in binding to ATP or the DnaA box sequence.

**W6A Is Defective in Self-oligomerization**—In a recent overlapping study, we examined the function of consecutive amino acids (Leu⁶, Trp⁶, Gln⁷, Gln⁸, and Cys⁹) in a predicted α-helix next to the N terminus and showed genetically that all except Gln⁷ are required for self-oligomerization (12). We also presented preliminary evidence that W6A is defective in self-oligomerization in a glutaraldehyde cross-linking assay. To extend these observations, we investigated the experimental conditions that are important to measure self-oligomerization and describe these results below. We found that cross-linked complexes of wild type DnaA were detectable in buffer supplemented with 0.1 M sodium chloride but not with 0.3 M or greater (Fig. 6 A). In 0.2 M sodium chloride, cross-linking of DnaA/H11001 increased with incubation time, but this was much less so with W6A (Fig. 6B). These results clearly indicate that tryptophan 6 functions in self-oligomerization. However, its substitution by alanine reduces but does not abolish cross-linking, prompting us to test whether other residues located in the N-terminal region account for the residual level of cross-linking. When we examined a truncated DnaA lacking the N-terminal 62 amino acids, we detected a negligible amount of cross-linked protein between the 203- and 303-kDa markers or above the latter, compared with those complexes observed with DnaA+ (Fig. 6C). The paucity of cross-linked DnaAΔ62 contrasts with the complexes formed by W6A, albeit at a reduced rate. Thus, it appears that...
Prepriming complexes were assembled on an oriC plasmid (M13oriC2LB5) as described previously (6) but with 0.2 µg of HU, 0.6 µg of DnaA+, or W6A, and no SSB. After isolation by gel filtration chromatography (Sepharose 4B; Amersham Biosciences), the amount of DNA in fractions corresponding to the void volume was quantified in ethidium bromide-stained agarose gels. Known amounts of M13oriC2LB5 DNA were co-electrophoresed in parallel to prepare a standard curve. The amounts of DnaA, W6A, and DnaB protein in the void volume fractions were measured in quantitative enzyme-linked immunosorbent assays. As a control, we substituted M13mp19 DNA for M13oriC2LB5 DNA under the above conditions; DnaB was not detectable in void volume fractions, and the level of nonspecific binding of DnaA+ corresponded to a ratio of 1.4 monomers per DNA. This background value has been subtracted to determine the stoichiometries of DnaA+ and W6A bound to M13oriC2LB5 DNA. The standard deviation and the range in the ratios of DnaA and DnaB per oriC plasmid from identical sets of experiments are also indicated. DnaC was not quantified because the experimental conditions include ATP, which, when hydrolyzed by DnaC, permits the release of DnaC from the prepriming complex (33).

### Table I

| Protein | DnaA | DnaB |
|---------|------|------|
| No. of experiments | Monomer/oriC | Range | No. of experiments | Monomer/oriC | Range |
| DnaA+ | 3 | 6.2 ± 0.8 | 5.3–6.8 | 3 | 10.5 ± 1.2 | 9.1–11.3 |
| W6A | 3 | 1.8 ± 0.5 | 1.3–2.3 | 3 | 1.8 ± 0.3 | 1.6–2.1 |

W6A Fails to Retain DnaB at oriC—Because the loading of DnaB at oriC requires that DnaA first oligomerizes at this locus (19), the results of Fig. 6 predict that W6A should fail to retain DnaB helicase at oriC. To test this prediction, we incubated W6A, DnaB, and DnaC with an oriC plasmid under conditions optimal to form the prepriming complex. DnaC must accompany DnaB in the DnaB-DnaC complex to assemble the prepriming complex, after which DnaC departs (33). To measure formation of the prepriming complex by the retention of DnaB on the oriC plasmid, we separated the plasmid DNA and proteins bound to it from unbound proteins by gel filtration chromatography and performed quantitative enzyme-linked immunosorbent assays on the isolated complex (Table I). We calculated a ratio of about two W6A monomers and near background levels of DnaB per oriC plasmid, indicating that the mutant protein’s defect in self-oligomerization leads to the failure to recruit DnaB to oriC. When we assembled the prepriming complex with DnaA+ instead, the ratio of about six DnaA monomers per plasmid DNA is generally consistent with previous results and is lower than the expected ratio of 10 monomers per oriC (6, 19). The ratio of close to 11 DnaA monomers per plasmid DNA substantiates observations that two DnaB hexamers are positioned at oriC, one for each replication fork to sustain bidirectional DNA replication (5, 6, 19).

We also measured the formation of a highly negatively supercoiled oriC plasmid, termed Form I*, which reflects the assembly of the prepriming complex and the subsequent movement of DnaB from oriC. Once loaded at oriC, DnaB helicase can unwind the oriC plasmid. In the presence of DNA gyrase to remove the positive superhelicity that accumulates in the duplex portion of the plasmid, a highly negatively supercoiled DNA results, which can be detected by agarose gel electrophoresis. On comparison of W6A to DnaA+, we found that the mutant was far less active (Fig. 7). Thus tryptophan 6 functions in self-oligomerization, which is required to load DnaB at oriC.

**DISCUSSION**

_Mutational Analysis of the N-terminal Region of DnaA Protein—_Previous studies indicate that the N-terminal region of DnaA is essential, apparently needed both to retain DnaB at oriC (11) and to support the association between and/or among DnaA monomers (12). To explore whether these functions are separate or interdependent, we undertook a mutational approach by substituting alanine for conserved residues in the N-terminal region and then measuring oriC plasmid maintenance in a host strain lacking the chromosomal dnaA gene. The analysis identified mutant DnaAs that appeared to be inactive in initiation. At the onset, we were concerned that an alanine substitution may alter the structure of the respective mutant protein to increase its susceptibility to proteolysis, reducing its steady-state level to be insufficient to support initiation. Alternatively, proteolyzed forms, which retain a subset of functions, may fail to support initiation but interfere with the activity of the intact protein, thus complicating the interpretation of results. To address these concerns, we performed immunoblot analysis of the mutant DnaAs expressed in a dnaA null host (E. coli MS3898) and found that L17A was undetectable, and W6A, L17A, P28L, L38A, L40A and I59A were partially proteolyzed. For the latter set, we cannot determine whether their phenotypes result from the respective substitution or from proteolyzed forms that interfere with the activity of the intact protein. Proteolysis of W6A was strain-dependent because it was not observed in derivatives of E. coli C600, MC1061, or in BL21(DE3) from which it was purified after overproduction.1 Proteolysis of L17A and P28L also appeared to be strain-de-
Tryptophan 6 Is Required for DnaA Oligomerization

Tryptophan 6 Is Required for DnaA Oligomerization

We confirmed in Fig. 4 earlier observations that DnaA binds to the DnaA boxes within oriC in an ordered manner, which arises from the different affinities of DnaA (R4 > R1 > R2 > R3) for these sites (30). Although the high affinity DnaA boxes R1 and R4 are identical, DnaA interacts with flanking sequences that influence binding affinity (36), leading to preferential binding to R4 over R1. The remaining DnaA boxes are similar but not identical to explain their lesser affinities and surface plasmon resonance measurements of the affinities of DnaA to R4 (KD = 0.6 ± 0.2 nM) and R2 (KD = 5.0 ± 1.0 nM) support these results (37). We suggest that after DnaA binds sequentially to the DnaA boxes of oriC, it oligomerizes to form a specific stable complex.

Despite the strong affinities of DnaA to DnaA boxes R1 and R4, DnaA rapidly dissociates from them (37, 38). Thus, although the caging effect of the polyacrylamide gel (39, 40) appears to stabilize W6A bound to the individual DnaA boxes of oriC in gel mobility shift assays, self-oligomerization of DnaA is essential to retain DnaA at oriC and in the prepriming complex (Ref. 19 and this work). That DnaA self-oligomerizes correlates well with DnaA as a member of the AAA family of oligomeric ATPases (35). Although it is attractive to consider that the DnaA oligomer assembled at oriC is required to load DnaB, itself a hexameric oligomer, the loading of DnaB by DnaA bound to the DnaA box contained in a hairpin structure of a single-stranded DNA does not require the oligomeric form of DnaA (Ref. 19 and this work), so this replication system somehow bypasses this requirement.

REFERENCES

1. Fuller, R. S., and Kornberg, A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 5817–5821
2. McGarry, K. C., Ryan, V. T., Grimwade, J. E., and Leonard, A. C. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 2811–2816
3. Brangulii, D., and Kornberg, A. (1988) Cell 52, 743–755
4. Marszalek, J., and Kaguni, J. M. (1994) J. Biol. Chem. 269, 4883–4890
5. Fang, L., Davey, M. J., and O'Donnell, M. (1999) Mol. Cell 4, 541–553
6. Carr, K. M., and Kaguni, J. M. (2001) J. Biol. Chem. 276, 44919–44925
7. Bell, S. P. (2002) Genes Dev. 16, 659–672
8. Erzberger, J. P., Pirruccello, M. M., and Berger, J. M. (2002) EMBO J. 21, 4763–4773
9. Morgan, W. (2002) FEMS Microbiol. Rev. 26, 355–374
10. Kaguni, J. M. (2004) in Encyclopedia of Biological Chemistry (Lennarz, W. J., and Lane, M. D., eds) Vol. 1, pp. 761–766, Academic Press, Amsterdam
11. Sutton, M. D., Carr, K. M., Vicente, M., and Kaguni, J. M. (1996) J. Biol. Chem. 273, 34255–34262
12. Simmons, I. A., Felcjak, M., and Kaguni, J. M. (2003) Mol. Microbiol. 49, 849–858
13. Sutton, M. D., and Kaguni, J. M. (1997) J. Mol. Biol. 274, 546–561
14. Lee, Y. S., and Hwang, D. S. (1997) J. Biol. Chem. 272, 83–88
15. Felczak, M. M., and Kaguni, J. M. (2004) Mol. Microbiol. 49, 1419–1431
16. Hwang, D. S., and Kaguni, J. M. (1988) J. Biol. Chem. 263, 10623–10640
17. Felczak, M. M., and Kaguni, J. M. (2004) J. Biol. Chem. 279, 51156–51162
18. Braun, R. E., O'Day, K., and Wright, A. (1985) Cell 40, 159–169
19. Kaguni, J. M., and Kornberg, A. (1984) Cell 38, 139–140
20. Massai, H., Nomura, N., and Arai, K. (1990) J. Biol. Chem. 265, 15134–15144
21. Simons, I. A., and Kaguni, J. M. (2003) Mol. Microbiol. 47, 755–765
22. Roth, A., and Messer, W. (1995) EMBO J. 14, 2106–2111
23. Serban, T., and Yokoyama, S. (2003) EMBO J. 22, 3077–3085
24. Doran, K. S., Helinski, D. R., and Konieczny, I. (1999) J. Biol. Chem. 274, 17921–17923
25. Marszalek, J., Zhang, W., Hupp, T. R., Margulies, C., Carr, K. M., Cherry, S., and Kaguni, J. M. (1996) J. Biol. Chem. 271, 18535–18542
26. Sutton, M. D., and Kaguni, J. M. (1997) J. Mol. Biol. 271, 693–703
27. Mima, S., Makise, M., Koterasawa, M., Tsuichiya, T., and Mizushima, T. (2002) Biochem. J. 365, 881–887
28. Carr, K. M., and Kaguni, J. M. (2002) J. Biol. Chem. 277, 39815–39822
29. Roth, A., and Messer, W. (1995) EMBO J. 14, 2106–2111
30. Margulies, C., and Kaguni, J. M. (1996) J. Biol. Chem. 271, 17035–17040
31. Konieczny, I., Doran, K. S., Helinski, D. R., and Blasina, A. (1997) J. Biol. Chem. 272, 10777–10782
32. Sekimizu, K., Brangulii, D., and Kornberg, A. (1987) Cell 50, 259–265
33. Wahle, E., Lasken, R. S., and Kornberg, A. (1989) J. Biol. Chem. 264, 2469–2475
34. Sutton, M. D., and Kaguni, J. M. (1997) J. Biol. Chem. 272, 23017–23024
35. Neuwald, A. F., Aravind, L., Spouge, J. L., and Koonin, E. V. (1999) Genome Res. 9, 27–43
36. Fujikawa, N., Kurumizaka, H., Nureki, O., Terada, T., Shibrouz, M., Katayama, T., and Yokoyama, S. (2003) Nucleic Acids Res. 31, 2077–2086
37. Blasing, F., Weigel, C., Welznek, M., and Messer, W. (2000) Mol. Microbiol. 36, 557–569
38. Margulies, C. A. (1997) The Influence of IHF and FIS on the Ordered Binding of DnaA Protein to oriC, the E. coli Chromosomal Origin. Ph.D thesis, Michigan State University, East Lansing, MI
39. Vosken, K. M., and Fried, M. G. (1997) Anal. Biochem. 245, 85–92
40. Fried, M. G., and Liu, G. (1994) Nucleic Acids Res. 22, 5054–5059
41. Kaguni, J. M. (1997) Mol. Cells 7, 145–157
42. Jakimowicz, D., Majka, J., Messer, W., Speck, C., Fernandez, M., Martin, M. C., Sanchez, J., Schauwecker, F., Keller, U., Schrepf, H., and Zakrzewska-Czerwinska, J. (1998) Microbiology 144, 1281–1290
43. Seitz, H., Weigel, C., and Messer, W. (2000) Mol. Microbiol. 37, 1270–1279
44. Garner, J., and Crooke, E. (1996) EMBO J. 15, 3477–3485
An Essential Tryptophan of *Escherichia coli* DnaA Protein Functions in Oligomerization at the *E. coli* Replication Origin
Magdalena M. Felczak, Lyle A. Simmons and Jon M. Kaguni

*J. Biol. Chem.* 2005, 280:24627-24633.
doi: 10.1074/jbc.M503684200 originally published online May 5, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503684200

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 43 references, 19 of which can be accessed free at http://www.jbc.org/content/280/26/24627.full.html#ref-list-1