JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2388–2390
0021-9193/92/072388-03$02.00/0

Vol. 174, No. 7

Chromosome Partitioning in *Escherichia coli* in the Absence of Dam-Directed Methylation

DANIEL VINELLA, ALINE JAFFÉ, RICHARD D'ARI, MASAMICHI KOHIYAMA, AND PATRICK HUGHES*

Institut Jacques Monod, Centre National de la Recherche Scientifique, Université Paris 7, 2, Place Jussieu, 75251 Paris Cedex 05, France

Received 30 July 1991/Accepted 24 January 1992

***Escherichia coli dam* mutants, lacking the GATC DNA methylase, do not produce anucleate cells at high frequencies, suggesting that hemimethylation of the chromosome origin of replication, *oriC*, is not essential for correct chromosome partitioning.**

Chromosome replication and cell division are strictly coordinated during the growth of *Escherichia coli*. Initiation of replication and cell division occur at precise moments in the cell cycle, and the division event takes place at the cell center after correct positioning of the chromosomes. It has often been assumed that this latter event, termed chromosome partitioning, requires attachment of the chromosome to one or more points in the cell membrane, primarily because prokaryotes lack a visible mitotic apparatus, such as the spindle, which carries out chromosome partitioning in eukaryotes. The notion is supported by numerous reports of DNA-membrane complexes for both chromosomal and plasmid DNAs (21). In chromosomal-DNA–membrane complexes, the DNA was found to be enriched for *oriC*, the replication origin (8). Recently, Ogden and colleagues have gone one step further by showing that most of the *oriC* DNA in these complexes is hemimethylated (20). Hemimethylation occurs transiently in newly replicated DNA because methylation is a postreplicative process. In *E. coli*, a major form of DNA methylation is carried out by the Dam methylase, which methylates adenine residues in GATC sequences (16). Hemimethylation at *oriC* lasts for 8 to 10 min, unusually long compared with periods of less than 1 min observed elsewhere (3, 20). These results suggest that replicated origins associate with the cell membrane for about 10 min and then detach. This residence time in the membrane could be involved in chromosome partitioning.

Hemimethylation of GATC sequences plays a well-documented role in strand discrimination for correction of mismatches introduced during DNA replication (7). In addition, hemimethylation has been implicated in control of initiation. Hemimethylation at *oriC* blocks initiation, as shown by the failure of hemimethylated *oriC* plasmids to replicate in *dam* mutants (25). This inhibition is apparently due to sequestration of hemimethylated origins in the cell membrane, since the replication of hemimethylated *oriC* plasmids in vitro in soluble cell extracts is inhibited by the membrane fraction (14). Replication of unmethylated DNA is not significantly affected by the membrane fraction (14), as one might expect since *dam* mutants are viable. Flow cytometer analysis and density shift experiments with *dam* mutants have shown that replication initiation is asynchronous (2) and can occur throughout the cell cycle, not only at the normal cell age for initiation (1). Taken together, these results suggest that hemimethylation of *oriC* and its transient attachment to the membrane play a role in preventing the occurrence of multiple initiations at a single origin in the same replication cycle.

These results do not rule out a role for membrane-origin complexes in partitioning, for initiation and partitioning could both be controlled by origin sequestration in the membrane. It is relevant to pose the question of what happens with chromosome partitioning in *dam* mutants, in which the GATC DNA methylase is inactive and origin-membrane association should no longer be operative. To test the contribution of hemimethylation to correct partitioning, we looked for the presence of anucleate cells, products of partitioning errors, in exponentially growing populations of a *dam* mutant. The *dam* mutant used in this study was constructed by P1 transduction of *dam-13*::Tn*9* from GM2199 (17) into parental strain PB103, a tryptophan auxotroph (6). The transductants were verified to have a mutator phenotype because of their failure to correct replication errors specifically on the newly synthesized (unmethylated) strand after replication and to have unmethylated DNA, as determined by its insensitivity to *Dpn*I and sensitivity to *Mbo*I, restriction endonucleases which cut DNA specifically at methylated and unmethylated GATC sequences, respectively. Coulter counter analysis and microscope examination of growing cultures of *dam* mutants showed heterogeneous cell volume distributions with normal-size, elongated, and filamentous cells (Fig. 1). This could be due to induction of SOS-associated division inhibitor SfiA, since *dam* mutants are partially induced for the SOS response (24). We therefore constructed a *dam-13 sfiA100*::Tn*5* double mutant (5). The cell volume distribution of the double mutant was also heterogeneous and varied from one clone to another (Fig. 1C and D), suggesting that SfiA does not completely account for the partial cell division inhibition in the *dam* mutant. This type of SOS-independent division inhibition has been previously observed under conditions in which initiation or elongation of DNA synthesis or chromosome decatenation was defective (12, 19).

Fluorescence microscopy, combined with 4′,6-diamidino-2-phenylindole staining (10), was used to observe nucleoids in cells from the different mutant populations. Cultures with different growth rates were obtained by growing cells in Luria Broth (LB), minimal glucose medium containing Casamino Acids plus tryptophan (MM Glu CA), or minimal glucose medium plus tryptophan (MM Glu). The results presented in Table 1 show the percentages of anucleate cells

* Corresponding author.

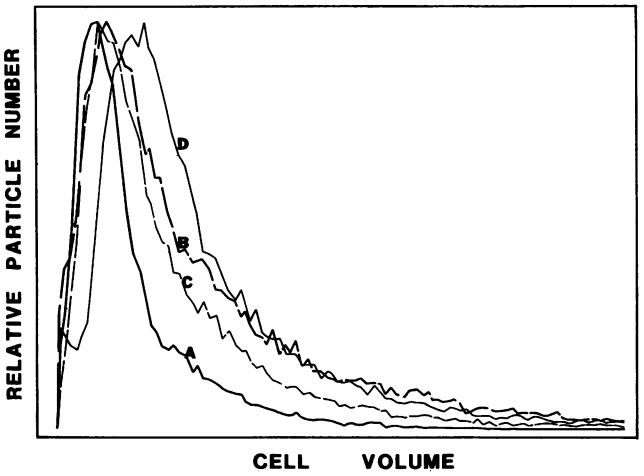

FIG. 1. Cell volume distributions of cultures grown in MM Glu at 37°C measured with a Coulter counter equipped with a C1000 Channelyzer. A, PB103; B, *dam-13*; C and D, *dam-13 sfiA-100*.

in cultures of two independently isolated *dam-13* transductants. The level of anucleate cells in *dam* mutants varied from one clone to another, with a tendency to increase at lower growth rates. The presence of an *sfiA* mutation had little effect. As a control, we used a *mutL218*::Tn*10* strain which, like the *dam* mutant, is defective in methyl-directed mismatch repair and has a mutator phenotype, although, unlike *dam*, it methylates GATC sequences in its DNA. In LB, the *dam* mutant has about the same level of anucleate cells as the *mutL* strain, approximately 0.2%. In minimal media, the *dam* mutant had somewhat higher levels than *mutL*, possibly as much as 10-fold, although the variability from clone to clone makes comparison difficult. In the latter media, some of the anucleate cells in the *dam* mutant may have resulted from lack of methylation. In the *dam* strains, we also observed filaments with diffuse nucleoids distributed along their length and elongated cells with a heavily stained DNA mass at the center (especially in the *sfiA* derivatives), reminiscent of mutants which fail to decatenate their replicated chromosomes (19).

TABLE 1. Frequency of anucleate cells in *dam*$^+$ and *dam-13* cultures grown in various media

Strain	Medium	% of anucleate cells[a]
PB103	LB	≤0.05
PB103	MM Glu CA	≤0.05
PB103	MM Glu	≤0.05
PB103 *dam-13*	LB	0.2, 0.1[b] (0.1)
PB103 *dam-13*	MM Glu CA	1.8, 1.6 (1.7)
PB103 *dam-13*	MM Glu	3.2, 1.0 (2.1)
PB103 *dam-13 sfiA100*	LB	2.1, 0.5, 0.6, 0.2[b] (0.8)
PB103 *dam-13 sfiA100*	MM Glu CA	1.1, 1.0, 1.2, 1.6 (1.2)
PB103 *dam-13 sfiA100*	MM Glu	4.7, 1.7, 0.8 (2.4)
PB103 *mutL218*[c]	LB	0.20
PB103 *mutL218*	MM Glu CA	0.20
PB103 *mutL218*	MM Glu	0.15

[a] From 500 to 2,000 individual bacteria from exponential-phase cultures growing at 37°C were analyzed for each sample. Numbers in parentheses are averages.

[b] Numbers refer to percentages of anucleate cells in two independently isolated *dam* transductants for PB103 *dam-13* and in four independent *sfiA100* transductants of one of these *dam-13* strains for PB103 *dam-13 sfiA100*.

[c] The *mutL218*::Tn*10* derivative was constructed by P1 transduction from GW3733 (23).

Many mutants initially suspected to be affected in partitioning turned out, in fact, to be defective in DNA primase or gyrase activity (11, 13, 19). Under nonpermissive conditions, mutants defective in DNA initiation, chain elongation, or chromosome decatenation produce anucleate cells and elongated cells with a centrally located nucleoid (19), like those observed in the *dam* strains. Recently, a partition mutant, *mukB*, was isolated and characterized (9, 18). In cultures of this mutant studied in the same experimental conditions and genetic background as in the present work, one occasionally sees unseparated daughter cells, one anucleate and the other with a double complement of nucleoids (18). Such images are not observed in cultures of *dam* strains.

On the basis of our microscope observations, we suggest that the aberrant cell division products in *dam* mutants are more likely to be due to a defect in DNA replication than to a defect in chromosome partitioning. We assume that origin-membrane interaction is severely affected in *dam* mutants. However, although the mutant has no hemimethylated DNA, there may be weaker interactions between unmethylated *oriC* sequences and, for example, the DnaA protein bound to the inner membrane surface (26). Our interpretation is in agreement with previous results showing that the *oriC* region of the chromosome is not sufficient for partitioning, since *oriC* plasmids are lost rapidly in the absence of selective pressure (22), although they initiate their replication in synchrony with the chromosome (15).

It is difficult to imagine a partitioning mechanism without interactions with the cell surface, since there should exist some point of anchorage for the partitioning apparatus. The MukB protein is a 177-kDa polypeptide whose predicted secondary structure resembles myosin or the kinesin heavy chain of eukaryotic cells (18). The deduced amino acid sequence of the amino-terminal part is homologous to that of a newly discovered protein, dynamin, a microtubule-associated mechanochemical enzyme found in bovine and rat brain tissues and in *Drosophila melanogaster* (4, 27). The MukB protein may provide the force required to move chromosomes to the correct position in the cell before division, suggesting that *E. coli* possesses cytoskeletal elements. The results presented here suggest that the partition recognition site on the bacterial chromosome is unlikely to be hemimethylated *oriC*.

We are grateful to Maury Fox and Miroslav Radman for useful discussions and to Philippe Bouloc and Denis Brachet for table and figure preparation.

This work was supported in part by the Association pour la Recherche sur la Cancer, contract 6696.

REFERENCES

1. **Bakker, A., and D. W. Smith.** 1989. Methylation of GATC sites is required for precise timing between rounds of DNA replication in *Escherichia coli*. J. Bacteriol. **171**:5738–5742.
2. **Boye, E., and A. Løbner-Olesen.** 1990. The role of *dam* methyltransferase in the control of DNA replication in *E. coli*. Cell **62**:981–989.
3. **Campbell, J. L., and N. Kleckner.** 1990. *E. coli* and the *dnaA* gene promoter are sequestered from *dam* methyltransferase following the passage of the chromosomal replication fork. Cell **62**:967–979.
4. **Chen, M. S., R. A. Obar, C. C. Schroeder, T. W. Austin, C. A. Poodry, S. C. Wadsworth, and R. B. Vallee.** 1991. Multiple forms of dynamin are encoded by *shibire*, a *Drosophila* gene

involved in endocytosis. Nature (London) **35:**583–586.
5. **D'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156:**243–250.
6. **de Boer, P., R. Crossley, and L. I. Rothfield.** 1989. A division inhibitor and a topological specificity factor coded for by the mini cell locus determine the proper placement of the division septum in *E. coli*. Cell **56:**641–649.
7. **Glickman, B. W., and M. Radman.** 1980. *Escherichia coli* mutator mutants deficient in methylation instructed DNA mismatch correction. Proc. Natl. Acad. Sci. USA **77:**1063–1067.
8. **Henrickson, W. G., T. Kusano, H. Yamaki, R. Balakrishnan, M. King, J. Murchie, and M. Schaechter.** 1982. Binding of the origin of replication of *Escherichia coli* to the outer membrane. Cell **30:**915–923.
9. **Hiraga, S., H. Niki, R. Imamura, T. Ogura, K. Yamanaka, J. Feng, B. Ezaki, and A. Jaffé.** 1991. Mutants defective in chromosome partitioning in *E. coli*. Res. Microbiol. **142:**189–194.
10. **Hiraga, S., H. Niki, T. Ogura, C. Ichinose, H. Mori, B. Ezaki, and A. Jaffé.** 1989. Chromosome partitioning in *Escherichia coli*: novel mutants producing anucleate cells. J. Bacteriol. **171:**1496–1505.
11. **Hussain, K., E. J. Elliott, and G. P. C. Salmond.** 1987. The *parD*$^-$ mutant of *Escherichia coli* also carries a *gyrAam* mutation: the complete sequence of *gyrA*. Mol. Microbiol. **1:**259–273.
12. **Jaffé, A., R. D'Ari, and V. Norris.** 1986. SOS-independent coupling between DNA replication and cell division in *Escherichia coli*. J. Bacteriol. **165:**66–71.
13. **Kato, J., Y. Nishimura, R. Imamura, H. Niki, S. Hiraga, and H. Suzuki.** 1990. New topoisomerase essential for chromosome segregation in *E. coli*. Cell **63:**393–404.
14. **Landoulsi, A., A. Malki, R. Kern, M. Kohiyama, and P. Hughes.** 1990. The *E. coli* cell surface specifically prevents the initiation of DNA replication at *oriC* on hemimethylated DNA templates. Cell **63:**1053–1060.
15. **Leonard, A. C., and C. E. Helmstetter.** 1986. Cell cycle-specific replication of *Escherichia coli* minichromosomes. Proc. Natl. Acad. Sci. USA **83:**5101–5105.
16. **Marinus, M. G.** 1987. DNA methylation in *Escherichia coli*. Annu. Rev. Genet. **21:**113–131.
17. **Marinus, M. G., M. Carraway, A. Z. Frey, L. Brown, and J. A. Arraj.** 1983. Insertion mutations in the *dam* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **192:**288–289.
18. **Niki, H., A. Jaffé, R. Imamura, T. Ogura, and S. Hiraga.** 1991. The new gene *mukB* codes for a 177 kDa protein with coiled-coil domains involved in chromosome partitioning of *E. coli*. EMBO J. **10:**183–193.
19. **Norris, V., T. Alliotte, A. Jaffé, and R. D'Ari.** 1986. DNA replication termination in *Escherichia coli parB* (a *dnaG* allele), *parA*, and *gyrB* mutants affected in DNA distribution. J. Bacteriol. **168:**494–504.
20. **Ogden, G. B., M. J. Prat, and M. Schaechter.** 1988. The replicative origin of the *E. coli* chromosome binds to cell membranes only when hemimethylated. Cell **54:**127–135.
21. **Ogden, G. B., and M. Schaechter.** 1986. The association of the *Escherichia coli* chromosome with the cell membrane, p. 45–49. *In* C. O. Gualerzi and C. L. Pon (ed.), Bacterial chromatin. Springer-Verlag KG, Berlin.
22. **Ogura, T., T. Miki, and S. Hiraga.** 1980. Copy-number mutants of the plasmid carrying the replication origin of the *E. coli* chromosome: evidence for a control region of replication. Proc. Natl. Acad. Sci. USA **77:**3993–3997.
23. **Pang, P. P., A. S. Lundberg, and G. C. Walker.** 1985. Identification and characterization of the *mutL* and *mutS* gene products of *Salmonella typhimurium* LT2. J. Bacteriol. **163:**1007–1015.
24. **Peterson, K. R., K. F. Westman, D. Mount, and M. G. Marinus.** 1985. Viability of *Escherichia coli* K12 DNA adenine methylase (*dam*) mutants requires increased expression of specific genes in the SOS regulon. Mol. Gen. Genet. **201:**14–19.
25. **Russell, D. W., and N. D. Zinder.** 1987. Hemimethylation prevents DNA replication in *E. coli*. Cell **50:**1071–1079.
26. **Sekimizu, K., B. Yat-Ming Yung, and A. Kornberg.** 1988. The DnaA protein of *Escherichia coli*: abundance, improved purification and membrane binding. J. Biol. Chem. **263:**7136–7140.
27. **Shpetner, H. S., and R. B. Vallee.** 1989. Identification of dynamin, a novel mechanochemical enzyme that mediates interactions between microtubules. Cell **59:**421–432.