JOURNAL OF BACTERIOLOGY, Oct. 1987, p. 4637–4645
0021-9193/87/104637-09$02.00/0

Vol. 169, No. 10

dnaA, an Essential Host Gene, and Tn*5* Transposition

JERRY C. P. YIN† AND WILLIAM S. REZNIKOFF*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received 6 February 1987/Accepted 17 July 1987

Mutations in *dnaA*, an essential gene in *Escherichia coli*, decrease the frequency of transposition of Tn*5*. An insertion mutation in the *dnaA* gene does not affect Tn*5* gene expression. Therefore, the DnaA protein plays a role either in the transposition reaction itself or in some type of cellular regulation of transposition. Analysis of a mutation in the DnaA box, found at the outside end of IS*50*, is consistent with a direct interaction of the protein through these bases. IS*50* transposition, which utilizes only one end containing a DnaA box, is not affected by *dnaA* mutations. Overproduction of the DnaA protein does not increase transposition frequencies in wild-type cells, even when the transposase is also overproduced.

Most procaryotic transposable elements encode a protein, the transposase, which is necessary for transposition of that element (27). Transposition requires cellular factors in addition to transposase. A complex of two small, histonelike proteins has been shown to be necessary for Mu transposition in vitro (10). This complex probably plays a mechanistic role in Mu transposition (10). Transposition of IS*1* and Tn*10* appears to require another complex of host proteins, the integration host factor (IHF), although it is not clear what role the IHF plays in IS*1* transposition (14; D. Morisato, personal communication). IHF consists of two small proteins with homology to the histone protein H1 (11, 26). IHF also is involved in the site-specific integration-excision of bacteriophage λ (26).

Tn*5* is a compound transposon. It consists of the insertion sequences IS*50*$_R$ and IS*50*$_L$, in an inverted orientation relative to one another, flanking a region that contains antibiotic resistance genes. The transposase is encoded by IS*50*$_R$ (35). When Tn*5* transposes, the outer ends of the compound transposon are used. The bases which constitute a minimal functional outer-end sequence have been defined (22). When the insertion sequence IS*50* transposes, one outer end and one inner end of IS*50* are used as the substrates. The sequence requirements at the inner end have also been examined (38).

Two host proteins have been implicated in Tn*5* transposition. DNA gyrase has been shown to be needed to keep the target molecule in the transposition reaction supercoiled (20). DNA polymerase I is also required, but only under certain experimental conditions (39, 44). It is unclear which of the functions of DNA polymerase I are needed for Tn*5* transposition (44).

The sequences at the outer end of Tn*5* that are necessary for transposition (22) contain homology to a repeated sequence in the *Escherichia coli* origin of replication (45). This sequence will be referred to as a DnaA box. Purified DnaA protein, the product of an essential host gene (17), binds to this sequence, both in the *E. coli* origin and at the outer end of Tn*5* (13).

In this paper we investigate the requirement for the DnaA protein in Tn*5* transposition in vivo. We show that it is required for full transposition proficiency. The DnaA protein does not affect the pattern of Tn*5* gene expression. Therefore, we postulate that it probably plays a direct role in the transposition reaction.

MATERIALS AND METHODS

Media, supplies, and general techniques. All bacteria were grown in LB broth or M9 medium supplemented with appropriate amino acids and vitamins (33). Antibiotic concentrations used were as follows: kanamycin, 40 μg/ml; tetracycline, 5 μg/ml; gentamicin, 5 μg/ml; chloramphenicol, 10 μg/ml; and ampicillin, 100 μg/ml. Antibiotics were purchased from Sigma Chemical Co. All radioactive compounds were from Amersham Corp. Restriction enzymes were from New England BioLabs, Inc., Bethesda Research Laboratories, Inc., or Promega Biotec. S1 nuclease and calf intestinal alkaline phosphatase were from Boehringer Mannheim Biochemicals. T4 polynucleotide kinase was from P-L Biochemicals, Inc. The large fragment of DNA polymerase I and T4 DNA ligase were gifts of M. Cox, F. Pugh, and B. Schutte. The recombinant DNA experiments were performed as described previously (31).

Strains, bacteriophages, and plasmids. All of the bacterial strains, bacteriophages, and plasmids used are described in Table 1. In general, antibiotic selection was maintained at all times for bacterial strains containing plasmids and insertions of transposons in the chromosome. Strains were constructed as described by Miller (33) except when noted.

The parental strain was D47.00 (CSH26 [33]). The RNase H::Tn*3* (18) marker was introduced by P1 transduction, selecting for ampicillin resistance. The *dnaA*::Tn*10* marker was moved by λ-mediated transduction as described previously (28). Tetracycline-sensitive derivatives were selected by the technique of Bochner et al. (7).

The *dnaA* mutants were monitored for the loss of function by a combination of techniques. (i) Tetracycline-sensitive derivatives were selected and shown to be incapable of maintaining pSC101. (ii) A fraction II crude extract was prepared and used to measure *oriC* replication programmed by an M13*oriC* template (13). The dependency on DnaA was shown by the addition of purified DnaA protein (kindly provided by R. Fuller). (iii) Southern blot analysis was performed as described previously (28), except that the plasmid pRB100 (8) was used as the probe.

* Corresponding author.
† Present address: Sino-American Biotechnology Company, Luoyang, Henan, People's Republic of China.

TABLE 1. Strains, phage, and plasmids used in this study

Strain, phage, or plasmid	Genotype or description	Source
Strains		
D47.00	Δ(*pro-lac*) *ara*	J. Miller
D47.20	D47.00 RNase H::Tn*3*	This study
D47.22	D47.20 *dnaA*::*850* Tn*10*	This study
D47.26	D47.22 tetracycline-sensitive derivative	This study
D47.30	D47.20 *ilv*::Tn*5*	This study
D47.32	D47.30 *dnaA*::*850* Tn*10*	This study
D47.33	D47.32 tetracycline-sensitive derivative	This study
D48.09	D47.00 *dam*::Tn*9* *ilv*::Tn*5* (λ84)	This study
D48.10	D47.20 *dam*::Tn*9*	This study
D48.15	D48.10 *dnaA*::*850* Tn*10*	This study
NS294	Δ*lacX74 bglR ilvO rpsL att*::P2II	A. Wright
NS366	NS294 *tna*::Tn*10 dnaA366*	A. Wright
DC204	Δ(*pro-lac*) *leu thy gly drm* Cyc^A *supE42* Hfr (81′) *tna*::Tn*10*	This study
DC204 *dnaA*	DC204 except *dnaA*::*850* Tn*10*	This study
I2.00	Hfl$^-$	B. Belfort
I68.01	*his galK* (λ *c*I857 Δ*B*am Δ*M*1 Δ8) *ilv*::Tn*5*/pOXgen	This study
I69.01	I68.01 except λ *c*I857 *N*7 *N*53 replaces λ *c*I857 Δ*B*am Δ*M*1 Δ8	This study
B7.21	D47.00 *rpsL rpsE gyrA polA* λ^r	R. Johnson
RZ211/F′I^q::Tn*5* (λ)	D47.00 *rpsL recA56*/F′ *pro lacI^q*::Tn*5* (λ *b*519 *b*515 *c*I857 *nin5 S*7)	R. Johnson
Phages		
λ84	λ *b*519 *b*515 *c*I857	M. Syvanen
λ299	λ *i*21 *nin5* (no *Eco*RI sites)	J. Way
λ500	λ *b*221 *c*I857 *O*29 *P*80 *rex*::Tn*5*	N. Kleckner
ColE1-based plasmids		
pRZ102	ColE1::Tn*5* (25)	R. Jorgenson
pRZ305	Protein fusion of p_1 and p_2 to β-galactosidase	R. Johnson
pRZ464	IS*50*$_R$ and the outer (1 to 15) base pairs of IS*50*$_L$ flanking tetracycline resistance (22)	R. Johnson
pRZ466	IS*50*$_R$ and the outer (1 to 21) base pairs of IS*50*$_L$ flanking tetracycline resistance (22)	R. Johnson
pRZ466-11A	pRZ466 with a mutation in the *dnaA* box	R. Johnson
pRZ914	Derivative of pRZ102 which is fully functional and contains a gene which codes for gentamicin resistance	M. Krebs
pBR322-based plasmids		
pBR25	*lac* promoter driving functional *dnaA* gene (8)	R. Braun
pRB36	*lac* promoter driving nonfunctional *dnaA* gene (8)	R. Braun
pRB100	Clone of the functional *dnaA* gene (8)	R. Braun
pRB101	Clone of a nonfunctional *dnaA* gene (8)	R. Braun
pRZ1095	Gentamicin-resistant derivative of pRB100	This study
pRZ1096	Gentamicin-resistant derivative of pRB101	This study
pBF110	p_L-driven *dnaA* gene (13)	R. Fuller
pBF1509	p_L-driven *dnaA* gene (B. Funnell, personal communication)	B. Funnell
pRZ1073	Protein-defective derivative of IS*50* with an outer end and an inner end flanking tetracycline resistance	This study
pRZ1113	Protein-defective derivative of IS*50* with two outer ends flanking tetracycline resistance	This study
pRZ986	Derivative of pBR322::Tn*5* with the wild-type promoters in IS*50*$_R$ replaced by the λ p_R promoter. A defective element carrying the gene which codes for gentamicin resistance located *cis* to the transposase-coding region is complemented in the transposition assays.	M. Krebs
RSF1010-based plasmids		
RSF1010::Tn*5*	RSF1010 with Tn*5* transposed onto it	This study
pRZ1054	Transposase-defective derivative of RSF1010::Tn*5*	This study

Strains were made *dam* by growth of P1 phage on a strain which contained the Tn*9* insertion in the *dam* gene. Transductants were selected for chloramphenicol resistance and screened for loss of methylase function by examining digests of plasmid DNA with *Mbo*I.

pRZ102 and RSF1010::Tn*5* have been described previously (23, 25), as have pRB100, pRB101, pRB25, and pRB36 (8). pBF110 and pBF1509 were kindly provided by R. Fuller. pRZ1095 and pRZ1096 are derivatives of pRB100 and pRB101, respectively; a *Pst*I fragment carrying a gentamicin resistance gene (S. Kagan, Ph.D. thesis, University of Wisconsin, Madison, 1981) was cloned into the unique *Pst*I site in each plasmid. pRZ1054 is a derivative of RSF1010::Tn*5* which does not synthesize transposase because of an internal deletion in the coding region.

pRZ464 and pRZ466 have been described previously (22). pRZ466-11A is a derivative of pRZ466 which contains a single T-to-A transversion in the DnaA box at one end of the Tn*5* element. It was created by oligonucleotide-directed mutagenesis, followed by sequencing and subcloning of a fragment which contained the outer-end sequence of Tn*5* (R. Johnson, personal communication).

pRZ1113 is a derivative of pBR322::IS*50*$_R$. The inner end of IS*50*$_R$ in the parent plasmid was replaced with a small fragment which contained the outer-end sequences of IS*50*. A tetracycline resistance gene was then exchanged with DNA internal to IS*50*. The resulting plasmid carries a defective element comprising two outer ends of IS*50* flanking a gene which confers tetracycline resistance. pRZ1073 is very similar to pRZ1113, except that the original inner-end sequence of IS*50*$_R$ was left intact. This plasmid also carries a defective element; in this case an outer end and an inner end flank a gene which confers tetracycline resistance. pRZ914 is a deleted derivative of pRZ102 (M. Krebs, personal communication). It contains a functional Tn*5* element which encodes gentamicin resistance.

pRZ986 is a derivative of pBR322::Tn*5* which contains a functional Tn*5* with the transposase-coding region under λ p_R promoter control (M. Krebs, personal communication). This plasmid was used in the *cis* complementation experiment (see Table 5).

Transposition assays. The lambda infection assay measures the Tn*5* transposition frequency from λ500, a replication-defective, integration-defective lambda phage which carries wild-type Tn*5*. After absorption, cells were diluted in LB broth and allowed to grow at 37°C for the equivalent of two cell doublings. The mixture was then plated on LB agar plates containing kanamycin and incubated at 37°C for 24 h. The number of transposition events was measured by counting the number of kanamycin-resistant colonies present. The number of drug-resistant colonies was linear with the number of input phage particles. Transposition frequencies are the fraction of input plaque-forming particles which yield kanamycin-resistant colonies. The multiplicity of infection was adjusted to yield between 50 and 500 kanamycin-resistant colonies per plate. The standard variation was less than a factor of 3, and all numbers quoted represent the average of assays done at least in triplicate.

The steady-state assay is an assay in which λ84 is infected into cells which carry a transposon, and it serves as the target in the transposition event. Cells were grown to saturation in LB broth containing antibiotic and maltose (0.2%). Phage lysates were made by mixing a fresh plaque of the phage (containing ca. 10^6 phage) with freshly saturated cells, buffer, and molten agar. This mixture was poured onto TYE plates (33) and incubated for 8 to 16 h at 37°C. The lysates were collected and spun, and the titer of the supernatant was determined (yields were typically $\geq 10^{10}$ phage). The transposition frequency was performed by measuring the fraction of plaque-forming phage that had acquired the particular drug resistance marker on the transposon. This measurement was performed by mixing a constant number of plaque-forming phage (typically 10^7 to 10^8) with 5×10^8 I2.00 cells and plating on selective antibiotic-containing plates after 60 min of growth. The frequency of lysogeny under these conditions approached 40%. The number of antibiotic-resistant colonies was shown to be linearly related to the amount of input phage.

The lambda induction assay determines the frequency of transposition of Tn*5* onto a λ prophage as measured by the abundance of λ::Tn*5*-transducing phage per infectious unit after prophage induction. This assay has been described previously (23), and the measurement of λ::Tn*5* was determined as described above. This assay was used in one of the experiments to test the effect of overproducing the DnaA protein.

The mating-out assay determines the frequency of transposition onto an F factor as measured by the occurrence of antibiotic-resistant exconjugants subsequent to a bacterial mating experiment. The mating-out transposition assay was performed as described previously (23), except that the cells were grown at 34°C and the mating mixture was also incubated at 34°C. A 10-μl portion of a freshly saturated, washed culture of the male and female strains was mixed in 1 ml of broth and incubated with slow shaking for 6 to 12 h. The cells were diluted and plated on appropriate media. A pOXgen derivative of the F factor was used. The exconjugants were selected on LB agar plates containing 200 μg of spectinomycin per ml and 10 μg of gentamicin per ml. Cells containing transposition products were measured on LB agar plates containing 200 μg of spectinomycin per ml, 50 μg of nalidixic acid per ml, 5 μg of gentamicin per ml, and 40 μg of kanamycin per ml. Strain B7.21 was used as the female strain in all matings.

The mating mixtures of male and female cells were diluted and plated to yield approximately 50 to 500 colonies per plate. The variation within a set of assays was usually less than twofold. All assays were performed in triplicate on a given day, and the numbers quoted represent averages of one or two sets of assays.

S1 nuclease mapping. RNA was isolated from strains D47.20, D47.22, D48.10, and D48.15 carrying pRZ102. The S1 mapping procedure, probes, and polyacrylamide gel analysis were identical to those described elsewhere (J. C. P. Yin, M. P. Krebs, and W. S. Reznikoff, J. Mol. Biol., in press).

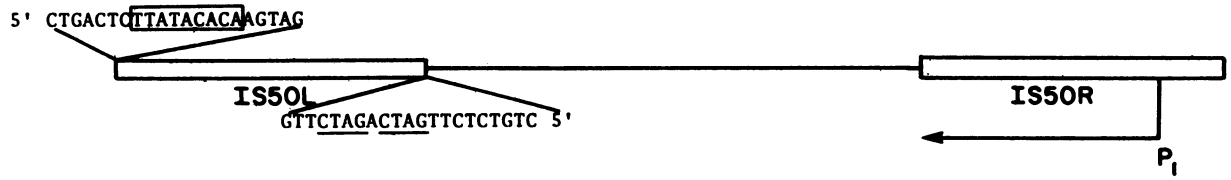
RESULTS

***dnaA* mutations and Tn*5* transposition.** Figure 1 shows the features of Tn*5* that are known to play a direct role in the transposition process. The transposase (p_1) is encoded by IS*50*$_R$ (35). Twenty-one base pairs at the outer end of IS*50*$_L$ and all of IS*50*$_R$ are sufficient for transposition of Tn*5* (22). Within the required 21 base pairs is a sequence very similar to a sequence that appears four times in the *E. coli* origin of replication (45). This sequence in IS*50* and *oriC* has been shown to be a binding site for the DnaA protein in vitro (12).

To determine whether *dnaA* is required for transposition of Tn*5* in vivo, transposition frequencies were measured in a series of *E. coli* strains with mutations in the *dnaA* gene. For these *dnaA* mutants to grow, they must contain an extragenic suppressor of the *dnaA* mutation (2, 27, 28, 32). Three different suppressors of *dnaA* lethality were used: the *rnh*::Tn*3* mutation and either an R factor or phage P2 as an integrative suppressor (2, 27, 28, 32). Table 1 lists the relevant strains, mutations, and suppressors. Three independent assays (see Materials and Methods) were used to confirm the lack of functional DnaA protein in the appropriate strains.

The data in Table 2 show the effect of mutations in the *dnaA* gene on Tn*5* transposition. An insertion of Tn*10* in the *dnaA* structural gene decreased transposition 25-fold when compared with transposition in a wild-type strain (lines 1 and 3). There was a 13-fold decrease in transposition between a RNase H mutant and the otherwise isogenic *dnaA*$^+$ strain (lines 2 and 3). RNase H mutants are extragenic suppressors of *dnaA* mutations which allow secondary replication origins to be used in a *dnaA*-independent manner (28). The RNase H mutation by itself had a negligible effect on the Tn*5* transposition (lines 1 and 2).

The following results (Table 2) support the conclusion that *dnaA* directly affects Tn*5* transposition. First, mutations in *dnaA* decreased the transposition frequency, regardless of



OUTER END 5' CTGACTCTTATACACAAGTAG

INNER END 5' CTGtCTCTTgatCAgAtcTtG

oriC REPEATS #1 TTATCCACA

#2 TTAT$^{A}_{G}$CACA

#3 TTATCC$^{A}_{C}$AA

#4 TTATCCACA

FIG. 1. Tn*5* sequence. The corresponding outer-end and inner-end sequences of IS*50* are shown and compared with the repeat sequences found in the *oriC* region of *E. coli*. The *dnaA* sequence present in the outer end is boxed, while the *dam* methylation sequences in the inner end are underlined. The transposase protein is shown diagramatically.

the site from which Tn*5* transposes. Transposition of Tn*5* from the multicopy plasmids pRZ102 and RSF1010::Tn*5*, which are derivatives of ColE1 and RSF1010, was affected by mutations in the *dnaA* gene (lines 2 and 3; lines 4 and 5). When Tn*5* transposed from the bacterial chromosome, *dnaA* also affected its transposition frequency (lines 6 to 8).

Second, the effect of *dnaA* can be demonstrated by using other transposition assays. The data presented in lines 1 to 8 were collected by using the steady-state assay. In this assay, lambda phage, which serves as the target in the transposition reaction, is grown lytically in cells which contain Tn*5*. Lines 9 and 10 show results obtained by using a λ donor assay in which transposition from an infecting phage molecule onto the bacterial chromosome is measured (24). In both the steady-state assay (lines 1 to 8) and the infection assay (lines 9 and 10), transposition was decreased in the *dnaA* mutants. We do not know whether there is any biological significance in the greater effect of the *dnaA* mutation in the infection assay than in the steady-state assay. In other experiments, *dnaA* mutations were found to have the same qualitative effect on Tn*5* transposition as indicated above when examined by a third assay, the lambda induction assay (data not shown).

Third, the decrease in transposition frequency occurred with other suppressors of the *dnaA* mutation. The transposition assays were repeated with an integrated R factor (lines 11 and 12) or a lysogen of phage P2 (lines 13 and 14) to suppress the *dnaA* mutation (30, 34). In both suppressor backgrounds, Tn*5* transposition decreased in the presence of a mutation in the *dnaA* gene.

Fourth, different null alleles of *dnaA* had similar effects on transposition. The strain which uses P2 as an integrative suppressor contains an amber mutation in the *dnaA* gene (40) instead of the Tn*10* insertion. Since this mutation still

TABLE 2. *dnaA* mutations and transposition

Strain	Relevant genotype	Suppressor	Starting location of Tn*5*	Transposition frequency	Relative frequency
1. D47.00	Wild type	None	pRZ102	$(3.9 \pm 2.0) \times 10^{-7}$	1.0
2. D47.20	*rnh*::Tn*3*	RNase H	pRZ102	$(2.1 \pm 1.3) \times 10^{-7}$	0.54
3. D47.22	*rnh*::Tn*3* *dnaA*::Tn*10*	RNase H	pRZ102	$(1.5 \pm 0.8) \times 10^{-8}$	0.04
4. D47.20	*rnh*::Tn*3*	RNase H	RSF1010::Tn*5*	$(4.4 \pm 0.3) \times 10^{-7}$	1.0
5. D47.22	*rnh*::Tn*3* *dnaA*::Tn*10*	RNase H	RSF1010::Tn*5*	$(1.1 \pm 0.9) \times 10^{-8}$	0.03
6. D47.30	*rnh*::Tn*3*	RNase H	*ilv*::Tn*5*	$(1.2 \pm 0.2) \times 10^{-7}$	1.0
7. D47.32	*rnh*::Tn*3* *dnaA*::Tn*10*	RNase H	*ilv*::Tn*5*	$(1.5 \pm 0.9) \times 10^{-8}$	0.13
8. D47.33	*rnh*::Tn*3* *dnaA*::Tn*10* Tet^{s}	RNase H	*ilv*::Tn*5*	$(1.7 \pm 1.5) \times 10^{-8}$	0.14
9. D47.20	*rnh*::Tn*3*	RNase H	Lambda	$(1.2 \pm 0.2) \times 10^{-4}$[a]	1.0
10. D47.22	*rnh*::Tn*3* *dnaA*::Tn*10*	RNase H	Lambda	$(1.0 \pm 0.1) \times 10^{-7}$[a]	<0.01
11. DC204	Hfr (81′) *tna*::Tn*10*	R100.1	pRZ102	$(8.4 \pm 3.6) \times 10^{-6}$	1.0
12. DC204^{-}	Hfr (81′) *dnaA*::Tn*10*	R100.1	pRZ102	$(9.2 \pm 5.8) \times 10^{-7}$	0.11
13. NS294	*att*::P2II	P2	pRZ102	8.4×10^{-6}[b]	1.0
14. NS366	*att*::P2II *dnaA366*(Am)	P2	pRZ102	2.2×10^{-8}[b]	<0.01

[a] The lambda infection assay was used to measure transposition. All other assays were the steady-state assay.
[b] Average of duplicate experiments. All other values are the averages of three or more experiments.

TABLE 3. *dnaA trans* complementation

Strain	Chromosomal genotype	Plasmid[a]	Gene on plasmid	Transposition frequency[b]
D47.20	*dnaA*$^+$	pRZ1095	*dnaA*$^+$	$(1.5 \pm 0.5) \times 10^{-6}$
D47.20	*dnaA*$^+$	pRZ1096	*dnaA*	$(1.7 \pm 0.1) \times 10^{-6}$
D47.22	*dnaA*	pRZ1095	*dnaA*$^+$	$(1.1 \pm 1.0) \times 10^{-6}$
D47.22	*dnaA*	pRZ1096	*dnaA*	$(1.0 \pm 0.3) \times 10^{-7}$

[a] All cells carry the plasmid RSF1010::Tn*5*, which is the source of Tn*5* in the transposition assay. Cells also carry a compatible coresident plasmid which contains a cloned copy of a functional or nonfunctional *dnaA* gene.
[b] The steady-state assay was used to measure transposition.

affected Tn*5* transposition (lines 13 and 14), the effect of different *dnaA* null alleles on transposition appears to be similar.

From these results we conclude that mutations in *dnaA* decrease the frequency of Tn*5* transposition. This effect is independent of the starting location of Tn*5*, the transposition assay used, the second-site suppressor used to permit cell growth, and the particular null allele of the *dnaA* gene.

The *dnaA* mutation does not affect Tn*5* transposition indirectly by affecting λ absorption. This possibility is ruled out by the following evidence. First, *dnaA* had the same qualitative effect on Tn*5* transposition by the λ induction assay (data not shown). This assay measures transposition onto an induced prophage and is independent of any possible adsorption problems. Second, Tn*3* and Tn*10* transposition showed no *dnaA* sensitivity by the same set of assays (data not shown). Third, phage growth was consistently better in the *dnaA* mutant background, an unlikely result if there were an absorption defect.

Control for polarity. Since the *dnaA* gene is part of an operon (36), inactivation of the structural gene through either an insertion mutation or an amber mutation may result in polarity on the expression of a distal gene such as *dnaN*. Therefore, we determined whether the effect of the *dnaA* mutation on Tn*5* transposition was due to the inactivation of the *dnaA* gene itself or to a possible polar effect on distal gene expression.

Transposition experiments were performed with wild-type or *dnaA* mutant cells which also contained plasmid clones of a functional or defective *dnaA* gene (8). Besides the functional or defective *dnaA* gene, the plasmids contained no other chromosomal genes. A cloned copy of the wild-type *dnaA* gene reversed the effect of a Tn*10* insertion in the chromosomal gene, while a cloned copy of an inactive *dnaA* gene did not (Table 3). This result shows that the effect of the *dnaA* mutation on Tn*5* transposition is primarily due to the inactivation of the *dnaA* gene itself.

Direct effect on Tn*5*. To show that the effect of the *dnaA* mutation on Tn*5* transposition is mediated by the DnaA box at the outer end of Tn*5*, the effect of a single point mutation in the *dnaA* consensus sequence was examined. One of the conserved bases in the sequence homology (46) was mutated to the sequence shown in Table 4. To assay the transposition of this mutant, we used compound elements which contain a wild-type IS*50* sequence and the outer-end sequences of IS*50*$_L$ (with or without the mutation). These sequences flank a selectable marker, the tetracycline resistance gene.

The effect of the DnaA box mutation on transposition is presented in Table 4. In cells containing the DnaA protein, the point mutation reduced transposition 50-fold (lines 1 and 2). The residual amount of transposition which the mutant shows was still above the background frequency of a deletion which removed one of the bases from the DnaA box (lines 2 and 3). In the absence of the DnaA protein, transposition with wild-type ends decreased eightfold (lines 1 and 4), as was also shown above (Table 2). In cells which lack *dnaA* and also contain a mutant DnaA box, transposition decreased an additional sixfold (lines 4 and 5). Thus, the DnaA box mutation decreased transposition whether or not the DnaA protein was present. In the presence of the mutation in the DnaA box, there was no additional effect when the DnaA protein was removed by mutation (lines 2 and 5).

The effect of the DnaA box mutation (50-fold), even when present on only one end of the element, was greater than the effect of removing the DnaA protein (8-fold). The simplest interpretation of these results is that the single-base change in the DnaA box affects the participation of two different components of the transposition machinery, the DnaA protein and an additional factor or factors. Presumably the total effect of the mutation in the DnaA box is the product of the effect of removing the DnaA protein and the effect on the additional factor or factors.

***dnaA* and IS*50* transposition.** Since the DnaA box appears only in the outer-end sequences of IS*50* (Fig. 1), a reasonable question is whether transposition of IS*50* (which uses one outer end and one inner end) requires the DnaA protein. Experiments were performed with defective elements with either two outer ends or one outer end and one inner end as the substrates to be complemented in *trans* by a transposase donor.

Table 5, lines 1 and 3, shows that there was an eightfold decrease in the efficiency of complementation of an element with two outer ends when transposition was measured in a

TABLE 4. Effect of *dnaA* site mutation[a]

Strain	Relevant genotype	Plasmid	Element description	Transposition frequency[b]	Relative frequency
D47.20	*dnaA*$^+$	pRZ466	Wild type	$(2.3 \pm 1.7) \times 10^{-7}$	1.0
D47.20	*dnaA*$^+$	pRZ466-11A	Point mutation	$(4.0 \pm 2.0) \times 10^{-9}$	0.02
D47.20	*dnaA*$^+$	pRZ464	Deletion of part of *dnaA* box	$\leq 1.0 \times 10^{-9}$	<0.01
D47.26	*dnaA*	pRZ466	Wild type	$(2.8 \pm 1.6) \times 10^{-8}$	0.12
D47.26	*dnaA*	pRZ466-11A	Point mutation	$(4.0 \pm 2.0) \times 10^{-9}$	0.02
D47.26	*dnaA*	pRZ464	Deletion of part of *dnaA* box	$\leq 1.0 \times 10^{-9}$	<0.01

[a] Wild type 5′-CTGACTCTTATACACAAGTAG
466-11A CTGACTCTTAAACACAAGTAG
464 CTGACTCTTATACACCGGGAT
[b] The steady-state assay was used to measure transposition. ≤ denotes the frequency if there had been one transposition event on the transposition assay plate.

TABLE 5. *dnaA* and end usage

Strain	Relevant genotype	Transposase donor plasmid[a]	Target in complementation[b]	Transposition frequency[c]	Relative frequency
1. D47.20	*dnaA*$^+$	RSF1010::Tn*5*	pRZ1113 (outer-outer)	$(4.2 \pm 0.8) \times 10^{-8}$	1.0
2. D47.20	*dnaA*$^+$	pRZ1054	pRZ1113 (outer-outer)	$\leq 4.7 \times 10^{-9}$	<0.1
3. D47.26	*dnaA*	RSF1010::Tn*5*	pRZ1113 (outer-outer)	$(5.4 \pm 4.6) \times 10^{-9}$	0.13
4. D47.26	*dnaA*	pRZ1054	pRZ1113 (outer-outer)	$\leq 2.0 \times 10^{-9}$	<0.1
5. D47.26	*dnaA*$^+$	RSF1010::Tn*5*	pRZ1073 (outer-inner)	$(3.4 \pm 2.4) \times 10^{-8}$	1.0
6. D47.20	*dnaA*$^+$	pRZ1054	pRZ1073 (outer-inner)	$\leq 2.0 \times 10^{-9}$	<0.1
7. D47.26	*dnaA*	RSF1010::Tn*5*	pRZ1073 (outer-inner)	$(2.0 \pm 2.0) \times 10^{-8}$	0.59
8. D47.26	*dnaA*	pRZ1054	pRZ1073 (outer-inner)	$\leq 2.0 \times 10^{-9}$	<0.1

[a] RSF1010::Tn*5* encodes a functional transposase, but pRZ1054 does not.
[b] The second, coresident plasmid contains the element to be complemented in *trans*. pRZ1113 contains an element that has two outer ends flanking a gene which codes for tetracycline resistance. pRZ1073 contains an element with an outer end and an inner end flanking a gene which codes for tetracycline resistance.
[c] The steady-state assay was used to measure transposition. ≤ denotes the frequency if there had been one transposition event on the transposition assay plate.

dnaA strain. However, when an element with an outer end and an inner end was examined, there was no significant decrease in the transposition frequency between a *dnaA*$^+$ and a *dnaA* strain (lines 5 and 7).

***dnaA* and Tn*5* gene expression.** Genetic evidence suggests a possible direct interaction between the DnaA protein and RNA polymerase (2, 3, 40). We therefore examined the possible role of such an interaction in the transcription of Tn*5* genes. Since the transposition frequency of Tn*5* has been shown to be a function of the concentration of both the transposase, p_1 (Yin et al., in press), and the inhibitor, p_2 (19, 24; submitted for publication), the DnaA protein might either stimulate expression of p_1 or depress expression of p_2. It has been shown previously that p_1 is translated from the T1 transcript, while p_2 is translated from the T2 transcript (29). Since the DnaA box is approximately 13 base pairs away from the putative −35 region of the T1 promoter and about 45 base pairs away from the −35 region of the T2 promoter (29), the DnaA protein could affect transcription from either promoter.

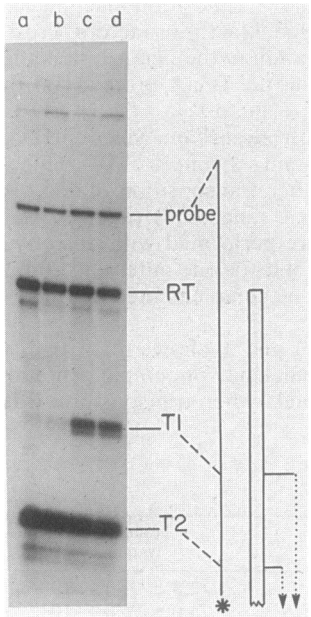


FIG. 2. S1 mapping of transcripts. An autoradiograph of an 8% polyacrylamide–7 M urea gel is shown. RNAs prepared from D47.20 (RNase H), D47.22 (RNase H *dnaA*), D48.10 (RNase H *dam*), and D48.15 (RNase H *dnaA dam*) cells containing plasmid pRZ102 correspond to lanes a to d, respectively. Two end-labeled probes were used in the S1 reactions, one of which hybridized to the β-lactamase gene (located in the chromosome) and the other of which detected Tn*5*-specific mRNA species. The relevant Tn*5* transcripts are shown to the right of the gel. RT denotes the readthrough transcript which enters the element from outside.

S1 mapping experiments on RNA isolated from either wild-type or *dnaA* mutant cells showed no difference in the pattern of protection for the two major transcripts from Tn*5*. Figure 2 is an autoradiogram of the gel from the S1 analysis. The levels of the T1 and T2 transcripts did not appreciably change in the mutant cells (compare lanes a and b). When the *dam* methylation mutation was added to these strains, there was still no effect of the *dnaA* mutation on the T1 or T2 transcripts (lanes c and d), although they showed the characteristic response to the methylation mutation (e.g., compare lanes a and c or lanes b and d).

Furthermore, the DnaA protein is required for Tn*5* transposition even when the transposase is expressed from a heterologous promoter. The dependence of transposition on *dnaA* was measured in cells in which transposase mRNA was placed under the control of the λ p_R promoter (M. Krebs, personal communication). The requirement of this element for the DnaA protein (Table 6, lines 3 and 4) was very similar to the requirement of the wild-type element (lines 1 and 2). Taken together, these two experiments show that *dnaA* does not affect Tn*5* transposition by affecting transcriptional initiation on the element.

Effect of overproducing DnaA. Since decreasing the amount of the DnaA protein decreases transposition, increasing the amount of the DnaA protein might increase the transposition frequency. Table 7 presents transposition data from various clones which overproduce the DnaA protein. Two of these clones place the *dnaA* gene under the control of the λ p_L promoter (13; R. Fuller, personal communication). Under fully inducing conditions, pBF110 overproduces the DnaA protein about 200-fold (13). The transposition experiments which involved these two clones were not performed under fully inducing conditions, nor was the amount of overproduction of the DnaA protein measured. The third clone which overproduces the DnaA protein places the *dnaA* gene under control of the lactose promoter (8). Transposition experiments with this overproducer were done in a strain deficient for the lactose repressor. Within the range of overproduction that these clones exhibit under these experimental conditions, there did not appear to be any stimulation of Tn*5* transposition. Some of the experiments presented in Table 7 were performed with *dam* mutants (lines 1

TABLE 6. Effect of *dnaA* when transposase is expressed from a heteologous promoter

Strain	Relevant genotype	Plasmid	Control of transposase synthesis	Transposition frequency	Relative frequency
D47.20	*dnaA*$^+$	pRZ914	Wild type	$(1.2 \pm 0.5) \times 10^{-7}$	1.0
D47.22	*dnaA*	pRZ914	Wild type	$(5.0 \pm 1.0) \times 10^{-9}$	0.04
D47.20	*dnaA*$^+$	pRZ986	$\lambda\ p_R$	$(3.3 \pm 0.3) \times 10^{-5}$[a]	1.0
D47.22	*dnaA*	pRZ986	$\lambda\ p_R$	$(3.7 \pm 0.7) \times 10^{-6}$[a]	0.11

[a] Steady-state transpositions were done onto λ299 and assayed at 34°C. All other transpositions were done onto λ84 and assayed at 37°C.

to 3), in which transposase levels are increased over wild-type levels (Yin et al., in press). Increasing the amount of the transposase still did not make the level of the DnaA protein rate limiting for transposition.

DISCUSSION

***dnaA* and Tn*5* transposition.** The experiments described in this paper involve the use of two different mutations in the *dnaA* gene (28, 40) to investigate the requirement for the DnaA protein in Tn*5* transposition. Both a Tn*10* insertion and an amber mutation result in a substantial decrease in transposition. The requirement for the DnaA protein in Tn*5* transposition is not absolute, since the decrease in the transposition frequency is only about 10-fold (Table 2). Mutations in the transposase or the sequences required at the outer end of Tn*5* have effects of at least 100-fold (22, 35).

Control experiments show that this effect is largely independent of the transposition assay, the type of suppressor which allows cell growth in the absence of the DnaA protein, and the donor site from which Tn*5* starts. A cloned copy of the *dnaA* gene reverses this effect, showing that directly or indirectly, the requirement is for the DnaA protein itself. Analysis of a single point mutation in the consensus DnaA box present at the outside end of Tn*5* is consistent with the interpretation that the DnaA protein interacts with this site. This genetic evidence, taken together with results of an in vitro binding experiment (12), suggests that *dnaA* exerts its effect on Tn*5* transposition by somehow acting through this site. Since transposition of IS*50* does not require the DnaA protein, the protein seems to function by interacting with the two DnaA box sequences located at each outer end of Tn*5*. An additional piece of evidence that supports this interpretation is that transposition of Tn*3*, Tn*9*, and Tn*10* is unaffected by mutations in the *dnaA* gene (data not shown). None of these elements contains DnaA box sequences.

The data indicate that mutations in the *dnaA* gene do not affect Tn*5* transcriptional initiation. There is no effect on the levels of the two major transcripts T1 and T2, nor is the amount of readthrough transcription affected. The complementation experiment presented in Table 6 is also consistent with the interpretation that mutations in *dnaA* have no effect on Tn*5* transcriptional initiation. Therefore, the requirement for the DnaA protein is probably not at the level of Tn*5* gene expression.

Role of *dnaA* in the cell. The DnaA protein is known to have two functions in the cell: repression of transcriptional initiation (8, 43; D. Smith, personal communication) and a role in the initiation of DNA replication (12, 15–17). Both of these functions are thought to involve binding of the protein at DnaA boxes, and binding with purified protein at some sites has been shown (8, 12). Is the requirement of the DnaA protein in Tn*5* transposition related to any of these other functions which *dnaA* is known to perform?

Repression. It is clear from the S1 mapping data and the complementation experiment presented in Table 6 that the *dnaA* mutation does not affect initiation of transcription on Tn*5*. The readthrough transcript which is detected in the S1 mapping experiment also is not affected by the *dnaA* mutation. Therefore, transcriptional elongation entering the element is probably not affected by the DnaA protein. Transcriptional fusion experiments which measure the amount of transcription traversing the DnaA box in either direction also show that *dnaA* has no effect (data not shown). However, the quality of the transcript, rather than the quantity, may be the important factor in Tn*5* transposition. It is possible that an RNA polymerase-DnaA protein complex is formed when RNA polymerase crosses the DnaA box and that this complex is the important species for Tn*5* transposition. There is some precedence for this hypothesis in the transcriptional

TABLE 7. Overproduction of DnaA

Strain	Relevant geneotype	Plasmid overproducer	Promoter/ gene	Transposition frequency
1. D48.09	*dam*	pBF110	$\lambda\ p_L$/*dnaA*$^+$	$(3.6 \pm 2.4) \times 10^{-5}$[a]
2. D48.09	*dam*	pRB25	*lac*/*dnaA*$^+$	$(6.6 \pm 4.2) \times 10^{-5}$[a]
3. D48.09	*dam*	pRB36	*lac*/*dnaA*	$(2.8 \pm 1.2) \times 10^{-5}$[a]
4. I68.01	*N*$^+$ host	None	None	3.1×10^{-5}[b]
5. I68.01	*N*$^+$ host	pBF110	$\lambda\ p_L$/*dnaA*$^+$	4.2×10^{-5}[b]
6. I68.01	*N*$^+$ host	pBF1509	$\lambda\ p_L$/*dnaA*$^+$	$(2.4 \pm 0.6) \times 10^{-5}$[b]
7. I69.01	*N* host	pBF1509	$\lambda\ p_L$/*dnaA*$^+$	$(2.6 \pm 1.8) \times 10^{-5}$[b]
8. RZ211/F'I^q (λ)	*recA*	pRB322	None	$(2.0 \pm 0.1) \times 10^{-6}$[a]
9. RZ211/F'I^q (λ)	*recA*	pRB25	*lac*/*dnaA*$^+$	$(6.3 \pm 2.7) \times 10^{-6}$[a]
10. RZ211/F'I^q (λ)	*recA*	pRB36	*lac*/*dnaA*	$(2.7 \pm 0.7) \times 10^{-6}$[a]

[a] The lambda induction assay was used to measure transposition.
[b] The mating-out assay was used to measure transposition.

activation model of *oriC* replication (43). We have no data to support or refute this model.

Replication. There is no direct evidence on whether extensive replication is needed by Tn*5* in the transposition process (6, 21). The elegant genetic evidence which shows that Tn*10* transposes by a conservative mechanism (4) does not exist for Tn*5*. The evidence which does exist suggests that Tn*5* does not transpose through an obligate, cointegrate structure as an intermediate (5, 21). The cointegrate is a replicative intermediate in the Shapiro model of transposition (41).

Interaction of ends. It is possible that the role of the DnaA protein in Tn*5* transposition is to bind to the DnaA box at each outer end and assist in properly aligning both ends in some type of nucleoprotein structure. Because the requirement for the DnaA protein in Tn*5* transposition is not absolute, it seems likely that the transposase by itself is also capable of bringing the two outer ends together, although not as efficiently. If this model is true, then IS*50* transposition, which is independent of *dnaA*, would rely on transposase by itself to align an outer end and an inner end in the proper structure.

A related possibility is that the DnaA protein facilitates the binding and/or function of transposase at the outer end. The spatial relationship between the presumed transposase-binding site, 5′-CTGACTCTT-3′, and the DnaA box is very interesting, since they actually overlap (Fig. 1). This overlap in binding sites is very similar to the relationship between the presumed transposase-binding site in IS*1* and the IHF-binding site (14). It is tempting to speculate that the two host factors may be performing analogous roles in the transposition process of these two elements.

Interaction of *dam* methylation and the DnaA protein. There is evidence that the *dam* methylation system and the DnaA protein probably interact. The DnaA protein represses expression of the *dam* gene (D. Smith, personal communication), and it has recently been shown that one of the two *dnaA* structural gene promoters is activated by *dam* methylation (9). Thus, these two genes and their gene products form an autoregulatory circuit. There is also a juxtaposition of DnaA boxes and *dam* methylation sites in the *oriC* region (45) and the *ori-r* region of the phage P1 (1). Both of these origins require *dnaA* to function. The *oriC* region also requires methylated bases to function (32, 42).

Transposition of IS*50* is sensitive to the *dam* methylation system by a mechanism which increases usage of the inner end as a substrate when it is undermethylated (Yin et al., in press). Usage of the two outer ends during Tn*5* transposition is independent of the *dam* methylation system (Yin et al., in press) but is stimulated by *dnaA*. It is possible that these two forms of stimulation, undermethylation acting on IS*50* inner ends and the DnaA protein acting on Tn*5* outer ends, are functionally analogous. For example, transposition may require the proper positioning of the two ends of the element together with the transposase. When IS*50* transposes, the transposase bound at the outer end and the inner end might bring the ends together to form this complex. Undermethylation of the inner end might stimulate the binding of the transposase to this site. When Tn*5* transposes, the DnaA protein bound at each outer end might bring the two ends together or facilitate the performance of this function by transposase. Thus, usage of either an outer end or an inner end might be facilitated by these two interacting genes and their products.

Cellular regulation of Tn*5* transposition. It is formally possible that the DnaA protein is involved in regulating the timing of Tn*5* transposition. The DnaA protein is known to be involved in the initiation of chromosomal replication (13, 17). This initiation event is a regulated process which occurs once per cell division. However, the DnaA protein is believed to be synthesized at a constant rate throughout the cell cycle (37). Therefore some form of temporal regulation exists which controls the timing of the initiation event, perhaps by controlling the activity of the DnaA protein. If transposition requires active DnaA protein, then transposition might occur within a limited part of the cell cycle.

ACKNOWLEDGMENTS

We thank M. Wickens for careful reading of the manuscript, M. Krebs and P. Lambert for discussions and aid, and A. Wright, R. Braun, R. Johnson, and T. Kogoma for strains. B. Fuller was very helpful in the initial stages of the work.

This work was supported by Public Health Service grant GM19670 from the National Institutes of Health. J.C.P.Y. was supported in part by Public Health Service Training Grant GM07133 from the National Institutes of Health.

LITERATURE CITED

1. **Abeles, A. L., K. M. Snyder, and D. K. Chattoraj.** 1984. P1 plasmid replication: replicon structure. J. Mol. Biol. **173:** 307–324.
2. **Atlung, T.** 1981. Analysis of seven *dnaA* suppressor loci in *Escherichia coli*. ICN-UCLA Symp. Mol. Cell. Biol. **22:** 297–314.
3. **Bagdasarian, M. M., M. Izakowska, and M. Bagdasarian.** 1977. Suppression of the DnaA phenotype by mutations in the *rpoB* cistron of ribonucleic acid polymerase in *Salmonella typhymurium* and *Escherichia coli*. J. Bacteriol. **130:**577–582.
4. **Bender, J., and N. Kleckner.** 1986. Genetic evidence that Tn*10* transposes by a non-replicative mechanism. Cell **45:**801–815.
5. **Berg, D. E.** 1983. Structural requirement for IS*50*-mediated gene transposition. Proc. Natl. Acad. Sci. USA **80:**792–796.
6. **Berg, D. E., and C. M. Berg.** 1983. The prokaryotic transposable element Tn*5*. Bio/Technology **1:**427–435.
7. **Bochner, B., H.-C. Huang, G. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
8. **Braun, R. E., K. O'Day, and A. Wright.** 1985. Autoregulation of the DNA replication gene *dnaA* in *Escherichia coli* K-12. Cell **40:**159–169.
9. **Braun, R. E., and A. Wright.** 1986. DNA methylation differentially enhances the expression of one of the two *E. coli dnaA* promoters *in vivo* and *in vitro*. Mol. Gen. Genet. **202:**246–250.
10. **Craigie, R., D. J. Arndt-Jovin, and K. Mizuuchi.** 1985. A defined system for the strand-transfer reaction at the initiation of bacteriophage Mu transposition. Proc. Natl. Acad. Sci. USA **82:**7570–7574.
11. **Flamm, E. L., and R. A. Weisberg.** 1985. Primary structure of the *hip* gene of *Escherichia coli* and of its product, the subunit of integration host factor. J. Mol. Biol. **183:**117–128.
12. **Fuller, R. S., B. E. Funnell, and A. Kornberg.** 1984. The *dnaA* protein complex with the *E. coli* chromosomal replication origin (*oriC*) and other DNA sites. Cell **38:**889–900.
13. **Fuller, R. S., and A. Kornberg.** 1983. Purified *dnaA* protein initiation of replication at the *Escherichia coli* origin of replication. Proc. Natl. Acad. Sci. USA **80:**5817–5821.
14. **Gamas, P., D. Galas, and M. Chandler.** 1985. DNA sequence at the end of IS*1* required for transposition. Nature (London) **317:**458–460.
15. **Hansen, E. B., and M. B. Yarmolinsky.** 1986. Host participation in plasmid maintenance: dependence upon *dnaA* of replicons derived from P1 and F. Proc. Natl. Acad. Sci. USA **83:**4423–4427.
16. **Hasunuma, K., and M. Sekiguchi.** 1977. Replication of plasmid pSC101 in *Escherichia coli* K12: requirement for *dnaA* function.

Mol. Gen. Genet. **154**:225–230.
17. **Hirota, Y., J. Mordoh, and F. Jacob.** 1970. On the process of cellular division in *Escherichia coli*. III. Thermosensitive mutants of *Escherichia coli* altered in the process of DNA initiation. J. Mol. Biol. **53**:369–387.
18. **Horiuchi, T., H. Maki, and M. Sekiguchi.** 1984. *RNaseH*-defective mutants of *Escherichia coli*: a possible discriminatory role of *RNaseH* in initiation of DNA replication. Mol. Gen. Genet. **195**:17–22.
19. **Isberg, R. R., A. L. Lazaar, and M. Syvanen.** 1982. Regulation of Tn*5* by the right repeat proteins: control at the level of the transposition reaction? Cell **30**:883–892.
20. **Isberg, R. R., and M. Syvanen.** 1982. DNA gyrase is a host factor required for transposition of Tn*5*. Cell **30**:9–18.
21. **Isberg, R. R., and M. Syvanen.** 1985. Tn*5* transposes independently of cointegrate resolution. J. Mol. Biol. **182**:69–78.
22. **Johnson, R. C., and W. S. Reznikoff.** 1983. DNA sequences at the ends of Tn*5* required for transposition. Nature (London) **304**:280–282.
23. **Johnson, R. C., and W. S. Reznikoff.** 1984. Role of the IS*50* proteins in the promotion and control of Tn*5* transposition. J. Mol. Biol. **177**:645–661.
24. **Johnson, R. C., J. C. P. Yin, and W. S. Reznikoff.** 1982. Control of Tn*5* transposition in *Escherichia coli* by a protein from the right repeat. Cell **30**:873–882.
25. **Jorgensen, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction enzyme cleavage map of Tn*5* and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177**:65–72.
26. **Kikuchi, Y., and H. A. Nash.** 1978. The bacteriophage λ int gene product. A filter assay for genetic recombination, purification of Int and specific binding to DNA. J. Biol. Chem. **253**:7149–7157.
27. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
28. **Kogoma, T., and K. von Meyenburg.** 1983. The origin of replication, *oriC*, and the *dnaA* protein are dispensable in stable DNA replication (*sdrA*) mutants of *Escherichia coli* K-12. EMBO J. **2**:463–468.
29. **Krebs, M. P., and W. S. Reznikoff.** 1986. Transcriptional and translational sites of IS*50*: control of transposase and inhibitor expression. J. Mol. Biol. **192**:781–791.
30. **Lindahl, G., Y. Hirota, and F. Jacob.** 1971. On the process of cellular division in *E. coli*: replication of the bacterial chromosome under control of prophage P2. Proc. Natl. Acad. Sci. USA **68**:2407–2411.
31. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Messer, W., U. Bellekes, and H. Lother.** 1985. Effect of *dam* methylation on the activity of the *E. coli* replication origin, *oriC*. EMBO J. **4**:1327–1332.
33. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Nishimura, A., Y. Nishimura, and L. Caro.** 1973. Isolation of Hfr strains from R^+ and $ColV2^+$ strains of *Escherichia coli* and derivation of an R'*lac* factor by transduction. J. Bacteriol. **116**:1107–1112.
35. **Rothstein, S. J., R. A. Jorgenson, K. Postle, and W. S. Reznikoff.** 1980. The inverted repeats of Tn*5* are functionally different. Cell **19**:795–805.
36. **Sakakibara, Y., and T. Mizukami.** 1980. A temperature-sensitive *Escherichia coli* mutant defective in DNA replication: *dnaN*, a new gene adjacent to the *dnaA* gene. Mol. Gen. Genet. **178**:541–553.
37. **Sakakibara, Y., and S. Yuasa.** 1982. Continuous synthesis of the *dnaA* gene product of *Escherichia coli* in the cell cycle. Mol. Gen. Genet. **186**:87–94.
38. **Sasakawa, C., G. F. Carle, and D. E. Berg.** 1983. Sequences essential for transposition at the termini of IS*50*. Proc. Natl. Acad. Sci. USA **80**:7293–7297.
39. **Sasakawa, C., U. Uno, and M. Yoshikawa.** 1981. The requirement for both DNA polymerase and 5′ to 3′ exonuclease activities of DNA polymerase I during Tn*5* transposition. Mol. Gen. Genet. **182**:19–24.
40. **Schaus, N. A., K. O'Day, and A. Wright.** 1981. Suppression of amber mutation in the *dnaA* gene of *Escherichia coli* K-12 by secondary mutation in *rpoB*. ICN-UCLA Symp. Mol. Cell. Biol. **22**:315–323.
41. **Shapiro, J.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci. USA **76**:1933–1937.
42. **Smith, D. W., A. M. Garland, G. Herman, R. E. Enns, T. A. Baker, and J. W. Zyskind.** 1985. Importance of state of methylation of *oriC* GATC sites in initiation of DNA replication in *Escherichia coli*. EMBO J. **4**:1319–1326.
43. **Stuitje, A. R., N. deWind, J. C. van der Spek, and M. Meijer.** 1986. Dissection of promoter sequences involved in transcriptional activation of the *Escherichia coli* replication origin. Nucleic Acids Res. **14**:2333–2344.
44. **Syvanen, M., J. D. Hopkins, and M. Clements.** 1982. A new class of mutants in DNA polymerase I that affects gene transposition. J. Mol. Biol. **158**:203–212.
45. **Zyskind, J. W., J. M. Cleary, W. S. A. Brusilow, N. E. Harding, and D. W. Smith.** 1983. Chromosomal replication origin from the marine bacterium *Vibrio harveyi* functions in *Escherichia coli*: *oriC* consensus sequence. Proc. Natl. Acad. Sci. USA **80**:1164–1168.