The EMBO Journal Vol.2 No.3 pp.463–468, 1983

The origin of replication, *oriC*, and the *dnaA* protein are dispensable in stable DNA replication *(sdrA)* mutants of *Escherichia coli* K-12

Tokio Kogoma* and Kaspar von Meyenburg

Department of Microbiology, The Technical University of Denmark, DK-2800 Lyngby-Copenhagen, Denmark

Communicated by K.von Meyenburg
Received on 18 January 1983

The *sdrA224* mutants of *Escherichia coli* K-12, capable of continued DNA replication in the absence of protein synthesis (stable DNA replication), tolerate inactivation of the *dnaA* gene by insertion of transposon Tn*10*. Furthermore, *oriC*, the origin of *E. coli* chromosome replication, can be deleted from the chromosome of *sdrA* mutants without loss of viability. The results suggest the presence of a second, normally repressed, initiation system for chromosome replication alternative to the 'normal' *dnaA*$^+$ *oriC*$^+$-dependent initiation mechanism.

Key words: oriC deletion/Tn*10* insertion in *dnaA*/secondary initiation system

Introduction

The replication of the *Escherichia coli* chromosome is regulated at the step of initiation. Initiation involves an interaction of a unique sequence *(oriC)*, located at 83.5 minutes of the *E. coli* chromosome, and the *dnaA* gene product. The products of four other genes, *dnaC, dnaI, dnaP* and *dnaB*, are also known to be involved in the process (for review, see Kornberg, 1980). The origin of replication, *oriC*, has been cloned and sequenced (Meijer *et al.*, 1979; Sugimoto *et al.*, 1979). The *oriC* has been shown to be the only functional replication origin in 'normal' *E. coli* strains (von Meyenburg and Hansen, 1980). The *dnaA* gene encodes a polypeptide with an apparent mol. wt. of 48 000–54 000 daltons (Hansen and von Meyenburg, 1979; Yuasa and Sakakibara, 1980). The gene has been cloned and sequenced (Hansen *et al.*, 1982b). Missense (temperature-sensitive or cold-sensitive) and nonsense (amber) mutations in this gene have been extensively characterized (Kornberg, 1980; Schaus *et al.*, 1981). Extragenic mutations *(das)* which suppress the defect in these mutants have been isolated and mapped in seven distinct loci (Bagdasarian *et al.*, 1977; Atlung, 1981). The *dnaA*$^+$-dependent initiation at *oriC* requires *de novo* protein synthesis and cannot continue in the absence of concomitant protein synthesis (von Meyenburg *et al.*, 1979).

Stable DNA replication *(sdrA)* mutants of *E. coli* are capable of repeated initiation despite the absence of protein synthesis (Kogoma, 1978). The stable DNA replication in these mutants is *recA*$^+$-dependent although DNA replication during the cell cycle (in the presence of protein synthesis) is not (Kogoma *et al.*, 1981; Torrey and Kogoma, 1982). Extragenic suppressor mutations *(rin)* which specifically suppress the defect of *recA* mutations in stable DNA replication (but not the recombinational or proteolytic functions of *recA*) have been isolated and mapped (Torrey and Kogoma, 1982). It has been proposed that *E. coli* has an alternative initiation pathway distinct from the *oriC*$^+$ *dnaA*$^+$-dependent initiation mechanism. Since mutations at the *sdrA* locus apparently allow for constitutive expression of the alternative mechanism (Kogoma, 1978), the product of the *sdrA*$^+$ gene may normally repress it. The alternative mechanism may involve a recombinational activity (Kogoma *et al.*, 1981; Torrey and Kogoma, 1982); furthermore, the induced stable DNA replication in wild-type cells may be due to a *recA*$^+$-dependent inactivation of the *sdrA*$^+$ gene product (Kogoma *et al.*, 1979).

The above proposal suggested that the second initiation pathway might be independent of the *oriC* sequence or the *dnaA* gene or both (Kogoma *et al.*, 1981). The construction of a specialized transducing λ phage carrying a deletion in the *oriC* region of the *E. coli* chromosome (von Meyenburg and Hansen, 1980) and of a λ phage carrying the *dnaA* gene inactivated by insertion of transposon Tn*10* (this work) has made it feasible to test the prediction directly.

Results

Suppression of dnaA^{ts} mutations by the sdrA mutation

The *E. coli* strains used in this work are listed in Table I. By P1-mediated transduction, eight different *dnaA* mutant alleles (*dnaA5, dnaA46, dnaA167, dnaA203, dnaA204, dnaA205, dnaA211* and *dnaA508*) were introduced into the *sdrA* mutant strain. The resulting *dnaA sdrA* double mutants grew at all temperatures in minimal medium, indicating that the *dnaA* mutations were suppressed in the *sdrA224* mutant. The suppression of the *dnaA167* and *dnaA508* mutations by a different stable DNA replication mutant, *sdrT*, in an *E. coli* 15T$^-$ strain has also been reported (Lark *et al.*, 1981). The degree of the suppression of the *dnaA* mutations by the *sdrA224* mutation was identical in all double mutants. Therefore, the suppression appeared not to be allele-specific. The presence of the *dnaA* mutations in the double mutants was ascertained by demonstrating that P1 phage grown on the double mutants co-transduced the temperature-sensitive phenotype with the *asnA* marker with a frequency of 10–25% (data not shown).

Figure 1 shows temperature-resistant DNA replication of the *dnaA46 sdrA224* double mutant at 42°C, contrasting with the complete cessation of DNA synthesis in the *dnaA46 sdrA*$^+$ counterpart. A similar result was obtained with the *dnaA5 sdrA224* mutant (data not shown).

The dnaA gene can be inactivated in sdrA mutants

λ*tna*406::Tn*10*-2420 carried the *dnaA* region of the *E. coli* chromosome which had a Tn*10* insertion in the *dnaA* gene (see Materials and methods; Figure 2a). Since the phage carried the *cI857* (temperature-sensitive phage repressor) mutation, infection of an *sdrA224 recA*$^+$ strain with the phage at 42°C in the presence of tetracycline was expected to select for Tet^R transductants as products of reciprocal recombination on both sides of the *dnaA* locus resulting in the replacement of the chromosomal *dnaA*$^+$ allele by the Tn*10*-inactivated

*To whom reprint requests should be sent. On leave from Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA (present address).

© IRL Press Limited, Oxford, England.

Table I. *E. coli* K-12 strains

Strains	Relevant genotype	Source, reference, construction
TC743	*dnaA46 tnaA600::*Tn*10*	CM734 (Hansen and von Meyenburg, 1979) xλ*tna*406::Tn*10*-2123 select Tet^R. T.Atlung
R2D2	*asnA31 asnB50::*Tn*5*	A.Wright (Felton *et al.*, 1980)
CM2123	a λ*tna*406::Tn*10*-2123/λ*cI857S7 b515 b519* lysogen of CM987	von Meyenburg and Hansen, 1980
CM2420	a λ*tna*406::Tn*10*-2420/λ*cI8577 b515 b519* lysogen of CM987	von Meyenburg and Hansen, 1980
AQ685[a]	*sdrA*$^+$ *metD88 proA3*	Derived from LS534; L.Soll
AQ694[a]	*sdrA224 proA3*	Derived from AQ685; this work
AQ699[a]	*sdrA224 metD88*	Derived from AQ685; this work
DK43	*sdrA224 dnaA5 ilv-192*	Derived from AQ694; this work
DK65	*sdrA*$^+$ *dnaA5 ilv-192*	Derived from AQ685; this work
DK81	*sdrA224 dnaA46 tnaA600::*Tn*10*	Pl(TC743) x AQ699, select Tet^R
DK101	*sdrA*$^+$ *dnaA46 tnaA600::*Tn*10*	Pl(TC743) x AQ685, select Tet^R
DK173	*sdrA224 dnaA5 ilv-192 asnB50::*Tn*5*	Pl(R2D2) x DK43, select Kan^R
DK175	*sdrA*$^+$ *dnaA5 ilv-192 asnB50::*Tn*5*	Pl(R2D2) x DK65, select Kan^R
DK209	*sdrA224 asnB50::*Tn*5*	P1(R2D2) x AQ699, select Kan^R
DK211	*sdrA*$^+$ *asnB50::*Tn*5*	Pl(R2D2) x AQ685, select Kan^R
DK274	*sdrA224 dnaA5 asnA31 asnB50::*Tn*5*	Pl(R2D2) x DK173, select Ilv$^+$
DK249	*sdrA224 dnaA850::*Tn*10*	This work
DK201	*sdrA224 asnB50::* Tn*5 dnaA5 ilv-192 oriC del-1071*	This work
DK241	*sdrA224 asnB50::*Tn*5 oriC del-1071*	This work

[a]The remaining genotype: *F*$^-$ *trpA9605 his-29 ilv*$^-$ *metB1 argH thyA deoB* or *C rpoB.*

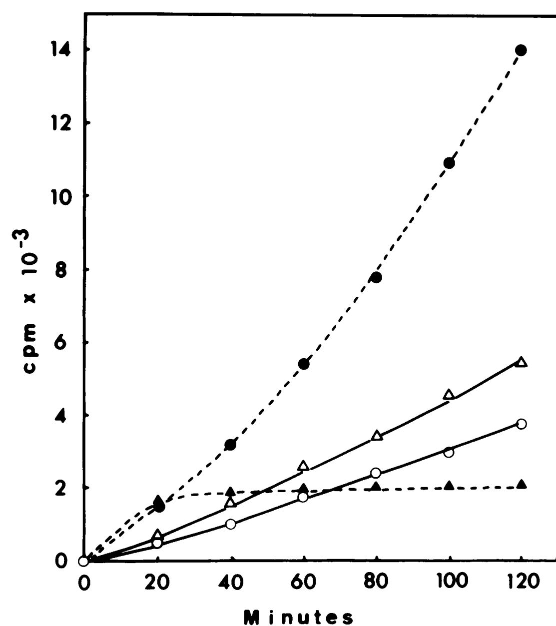

Fig. 1. Effect of high temperatures on DNA synthesis of *sdrA224 dnaA46* and *sdrA*$^+$ *dnaA46* strains. DK81 (*sdrA224 dnaA46*; circles) and DK101 (*sdrA*$^+$ *dnaA46*; triangles) were grown at 30°C to ~2 x 10^8 cells/ml. The cultures were then split into halves and incubated at 30°C (○, △) or 42°C (●, ▲) in the presence of [^{3}H]thymine (10 μCi/8 μg/ml). 100 μl samples were taken as indicated and treated as previously described (Kogoma, 1978). Growth media and growth conditions were described elsewhere (Kogoma, 1978).

dnaA allele if the *sdrA* mutation also suppresses the complete loss of the *dnaA* function. The results summarized in Table II show that infection of the *sdrA224* mutant (AQ699) with this phage yielded Tet^R transductants with a high frequency whereas the *sdrA*$^+$ counterpart (AQ685) gave rise to a few Tet^R colonies only: the latter were probably products of Tn*10* transposition or integration of λ reverted for the *cI857* mutation. When λ*tna*406::Tn*10*-2123, which had a Tn*10* insertion in the *tnaA* gene *(tnaA600::Tn10)* (Figure 2a) was used, both strains yielded Tet^R transductants with high frequencies (Table II).

Phage P1 was grown on one of the Tet^R transductants (DK249) obtained after transduction of AQ699 with λ*tna*406::Tn*10*-2420. Using the resulting P1 lysate, the transduction experiment was repeated. The results (Table III) indicated again that the *sdrA224* strain (AQ699) but not the *sdrA*$^+$ counterpart (AQ685) could tolerate the recombinational replacement of the *dnaA*$^+$ gene by the insertionally inactivated allele *dnaA850::*Tn*10*.

Evidence for the proper position of the *dnaA850::*Tn*10* allele in the chromosome of the Tet^R transductants was obtained in two ways. First, we could show linkage between *tet* and *asnA* in P1 transduction experiments using strain DK274 *(sdrA*$^-$*asnA*$^-$*asnB*$^-$*)* as the recipient (Felton *et al.*, 1980); a 24% co-transduction frequency was found (Table III) in good agreement with 10–25% co-transduction between *dnaA*^{ts} alleles and *asnA* found in other experiments (see above). Secondly, we could demonstrate physical replacement of the *dnaA*$^+$ allele by the *dnaA850::*Tn*10* allele by hybridization of an appropriate ^{32}P-labelled probe to *Eco*RI fragments of total chromosomal DNA from such Tet^R transductants. Most of the *dnaA*$^+$ gene sequence is located on a 3.6-kb *Eco*RI fragment (Hansen and von Meyenburg, 1979) between coordinates 0.95 and 4.45 in Figure 2a. The transposon Tn*10* in the *dnaA* gene of λ*tna*406::Tn*10*-2420 is located at coordinate 1.1 such that, when recombined into the chromosome, the 3.6-kb *Eco*RI fragment should be missing and two new fragments of 6.7 and 7.4 kb should be created [Tn*10* has one site for *Eco*RI (Jorgensen and Reznikoff, 1979)]. The hybridization of the chromosomal *Eco*RI fragments to the ^{32}P-labelled 3.6-kb fragment (from pJC605) revealed that the parental strain AQ699 (*dnaA*$^+$) contained the 3.6-kb fragment (Figure 2b lanes 4–5) whereas the Tet^R transductant DK249 did not (Figure 2b lanes 1–3). A 6.7-kb fragment was detected in DK249 as expected (Figure 2b lanes 1–3): the other new fragment of an expected size of 7.4 kb was not visualized, probably because that fragment carried only

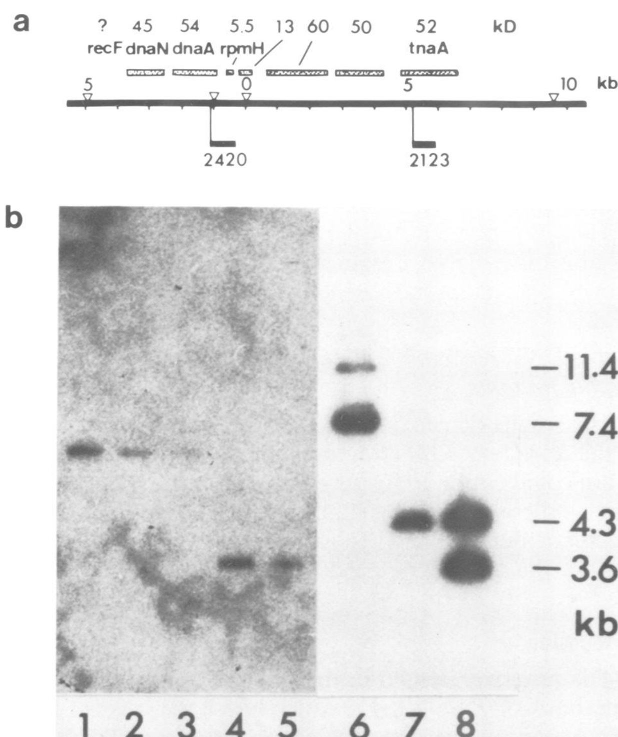

Fig. 2. a. Genetic and physical map of the *dnaA-tnaA* region of the *E. coli* chromosome. The locations of the *tnaA, rpmH, dnaA, dnaN* and *recF* genes are drawn according to Hansen and Von Meyenburg (1979) and Hansen *et al.* (1982a). The positions of the genes coding for the 13, 60 and 50 K proteins as determined by F.Hansen, E.B.Hansen and K.von Meyenburg (unpublished) are also included. Insertion sites and orientations of Tn*10* are indicated by the flag pointing away from the *tet* gene which are located asymmetrically on the transposon (Jorgensen and Reznikoff, 1979). ∇ indicates *Eco*RI sites. **b.** Autoradiogram of *Eco*RI fragments of DK249 and AQ699 after being hybridized to ^{32}P-labelled pJC605 plasmid containing the *dnaA* region. DNA was purified from DK249 *(sdrA224 dnaA850::*Tn*10)* and AQ699 *(sdrA224 dnaA*$^+$*)* and digested with *Eco*RI to completion. The *Eco*RI fragments were electrophoresed on an 0.7% agarose gel for 3 h together with *Eco*RI-digested reference DNA (see below) and then electrophoretically transferred onto a freshly prepared sheet of DBM paper. The paper was subjected to two-phase hybridization using a ^{32}P-labelled probe prepared by nick-translation of pJC605 (3.3 x 10^7 c.p.m./µg). pJC605 was a pBR322 derivative which contained a 3.6-kb *Eco*RI fragment of the *dnaA* region of the *E. coli* chromosome; **lanes 1–5** represent a portion of an autoradiogram obtained after 22 h of exposure; **lanes 6–8** after 2 h of exposure. **Lane 1,** DK249 *(sdrA224 dnaA850::*Tn*10)* DNA, 100 ng. **Lane 2,** DK249 DNA, 30 ng. **Lane 3,** DK249 DNA, 10 ng. **Lane 4,** AQ699 *(sdrA224 dnaA*$^+$*)*, 100 ng. **Lane 5,** AQ699, 30 ng. **Lane 6,** *Eco*RI-digested λ*tna*406::Tn*10*-2420 DNA: the 7.6-kb fragment is the terminal (left arm) fragment of the phage DNA joined to the left half of the Tn*10* inserted (see text). The 11.4-kb fragment represents the two terminal fragments joined through the cohesive ends. **Lane 7,** *Eco*RI digest of λ*tna*406::Tn*10*-2098 DNA which has a Tn*10* insertion in the *tnaA* gene like λ*tna*406::Tn*10*-2123; the 4.3-kb fragment, the left arm terminal fragment carrying most of the 3.6-kb *Eco*RI chromosomal DNA. **Lane 8,** *Eco*RI-digested pJC605; 3.6-kb, *dnaA*$^+$-containing chromosomal DNA; 4.3-kb, pBR322 vector DNA.

100–150 bp of chromosomal DNA homologous to the 3.6-kb probe whereas the 6.7-kb fragment carried ~3400 bp (E.B.Hansen and K.von Meyenburg, unpublished observation). The results show that the *dnaA850::*Tn*10* allele was indeed successfully transferred from phage λ*tna*406::Tn*10*-2420 into the chromosome of the *sdrA* strain replacing the *dnaA*$^+$ allele.

The oriC site can be deleted in sdrA mutants

The *oriC* sequence is located between *asnA* and *gidA*

Table II. Transduction of *sdrA224* and *sdrA*$^+$ strains to Tet^R by λ*tna*406 carrying *dnaA850::*Tn*10* or *tnaA600::*Tn*10*

Recipient strain	No. of Tet^R transductants at 42°C			
	λ*tna*406::Tn*10*-2420 *(dnaA850::*Tn*10)*		λ*tna*406::Tn*10*-2123 *(tnaA600::*Tn*10)*	
	10^0	10^{-1}	10^0	10^{-1}
AQ699 *(sdrA224)*	255	25	362	45
AQ *(sdrA*$^+$*)*	4	0	380	63

λ*tna*406::Tn*10*-2123 and λ*tna*406::Tn*10*-2420 lysates were prepared by heat induction of CM2123 and CM2420. Approximately 1 x 10^9 cells were infected with 0.1 ml of undiluted (10^0) and 10-fold diluted (10^{-1}) lysates. The titers of the undiluted lysates of λ*tna*406::Tn*10*-2420 and λ*tna*406::Tn*10*-2123 were 1.6 x 10^8 and 1.1 x 10^8 p.f.u. (helper phage)/ml, respectively.

Table III. Transduction of *sdrA224* and *sdrA*$^+$ strains to Tet^R by phage P1 lysates grown on the *dnaA850::*Tn*10* strain, DK249

Exp.	Recipient strain	No. of Tet^R transductants		
		m.o.i. = 1	m.o.i. = 0.1	%Asn$^+$
I	AQ699 *(sdrA224)*	142	6	–
	AQ685 *(sdrA*$^+$*)*	2	0	–
II	DK274 *(sdrA224 asnA*$^-$ *asnB*$^-$*)*	89	–	24

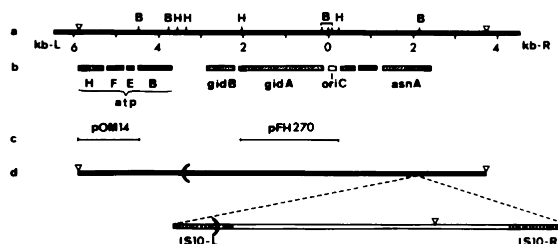

Fig. 3. Genetic and physical map of the *oriC* and *atp* operon region of the *E. coli* chromosome. **(a)** Restriction sites for the enzymes *Eco*RI (∇), *Bam*HI (B) and *Hin*dIII (H) (von Meyenburg *et al.*, 1982). **(b)** Positions of genes coding for proteins according to von Meyenburg *et al.* (1982). **(c)** Locations of the chromosomal DNA fragments carried by plasmids from which ^{32}P-labelled probes were prepared. **(d)** The site of the Tn*10* insertion in the chromosomal 9.8-kb *Eco*RI fragment. Approximate location and extent of the *oriC del-1071* (von Meyenburg and Hansen, 1980) is indicated by brackets.

(Figure 3) (von Meyenburg and Hansen, 1980; Hansen *et al.*, 1981). A specialized transducing phage λ carrying this region of the *E. coli* chromosome with a deletion extending from within the *asnA* gene to the end of the *gidB* gene including *oriC* had been constructed (λ*asn*132 *oriC del-1071*) (Figure 3: von Meyenburg and Hansen, 1980). This phage had been used to construct *E. coli* strains that lacked the entire *oriC* sequence. The construction of such *oriC* deletion strains was possible only in strains carrying an integrated F or R1 replicon (von Meyenburg and Hansen, 1980). The same phage was used to test whether or not the *oriC* site can be deleted in *sdrA* mutants. The *oriC* deletion *del-1071* in this phage genome is flanked by chromosomal DNA on the left side (counter-clockwise) and by Tn*10* DNA and adjacent chromosomal DNA on the right (clockwise). The flanking sequences were expected to allow recombination between this λ phage and the *oriC* region of the *E. coli* chromosome,

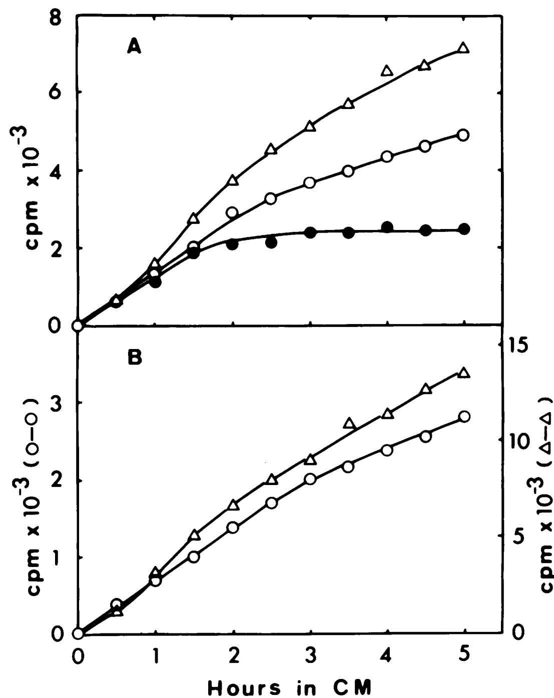

Fig. 4. Stable DNA replication in *sdrA224 dnaA::*Tn*10* and *sdrA224 ΔoriC* mutants. **A:** DK249 (*sdrA224 dnaA850::*Tn*10*, △), DK209 (*sdrA224 dnaA*$^+$ ○) and DK211 (*sdrA*$^+$ *dnaA*$^+$, ●) were grown at 37°C to 2 x 10^8 cells/ml. At time = 0 chloramphenicol (CM) (150 μg/ml) and [^{3}H]thymine (10 μCi/8 μg/ml) were added. **B:** DK201 (*sdrA224 oriC del-1071 dnaA5*, ○) and DK241 (*sdrA224 oriC del-1071*, △) were grown at 37°C. DNA synthesis in the presence of CM was measured as described in Figure 1.

resulting in replacement of a part of *asnA* and the entire region including *oriC gidA* and probably *gidB* by *del-1071* and the *tet* gene of Tn*10* (Figure 3D). Selection for such recombinants was, for Tet^R at 42°C, as in the experiments with λ*tna*406::Tn*10*-2420 described above. *asnB50::Tn5* strains were used so that resulting recombinants should exhibit an Asn$^-$ phenotype (Felton *et al.*, 1980). After infection of DK209 *(sdrA224 asnB50::*Tn*5)* with the *oriC* deletion phage, 15 slowly growing Tet^R transductants and a few fast ones were found: only the former were Asn$^-$. With the isogenic *sdrA*$^+$ strain (DK211) a few Tet^R transductants were found, all of which were *Asn*$^+$. A similar result was obtained using a pair of *dnaA5* strains (DK173, *sdrA224 asnB50::*Tn5, and DK175, *sdrA*$^+$ *asnB50::*Tn*5*) as recipients: the *sdrA*$^+$ recipient did not yield any Tet^R transductants whereas the *sdrA*$^-$ strain yielded 35 Tet^R transductants at 40°C, of which >95% were Asn$^-$. These results indicated that *oriC del-1071* could be efficiently transferred into the chromosome of *sdrA224* strains as previously demonstrated with integratively suppressed strains (von Meyenburg and Hansen, 1980).

The absence of the *oriC* sequence from these transductants of the *sdrA224* strain was verified, analogously to the visualization of the *dnaA850::*Tn*10* allele (see above), by hybridization of chromosomal *Eco*RI fragments to appropriate ^{32}P-labelled probes, i.e., pOMC14 carrying the *atpEFH* genes and pFHC270 carrying *oriC* and the adjacent *gidA* gene (Hansen *et al.*, 1981) (Figure 3C). The *oriC*-containing 9.8-kb *Eco*RI fragment (von Meyenburg and Hansen, 1980) was detected by both of the two probes in the chromosomal DNA from the *oriC*$^+$ strain DK173 (data not shown). The chromosomal DNA from DK201, one Tet^{R}Asn$^-$ transductant of the *sdrA224* strain (DK173), lacked the 9.8-kb fragment: instead, a new fragment of 7.6 kb was found hybridizing to the pOMC14 probe but not to pFHC270 (data not shown). This new fragment of 7.6 kb was expected on the basis of the size of the *Eco*RI fragment in the phage λ*asn*132 *oriC del-1071* (von Meyenburg and Hansen, 1980), which contained the *oriC* deletion (Figure 3D). The pFHC270 probe did not hybridize to a fragment of this size or any other DNA fragments of strain DK201 (data not shown), indicating the absence of the *oriC* sequence from this strain.

Stable DNA replication in dnaA::Tn10 sdrA and ΔoriC sdrA double mutants

The results depicted in Figure 4 show that DNA synthesis in the *ΔoriC sdrA224* strain and the *dnaA850::*Tn*10 sdrA224* strain could continue for several hours in the absence of protein synthesis. Thus, the stable DNA replication endowed by the *sdrA* mutation in these strains required neither the *oriC* site nor the *dnaA* gene product.

Discussion

The results presented in this paper demonstrate that *sdrA* mutations of *E. coli* K-12, which had been selected for conferring upon the cell the capability of continued DNA replication in the absence of protein synthesis, render the chromosome replication independent of the normal initiation system. Firstly, the *sdrA* mutations not only suppressed the temperature-sensitivity of a series of *dnaA* mutants but also allowed the disruption of the *dnaA* gene by insertion of transposon Tn*10*. Secondly, *oriC* deletion mutations could be transferred into the chromosome of the *sdrA* strains in the same way as into strains harboring an F or R1 replicon integrated in the chromosome (von Meyenburg and Hansen, 1980). These genetic manipulations were not possible in the *sdrA*$^+$ counterparts. Thus, the *sdrA* mutant can dispense with both the chromosomal origin of replication, *oriC*, and the *dnaA* gene, the product of which specifically acts as a positive initiation factor at *oriC* (von Meyenburg *et al.*, 1979). The *sdrA* mutations which resulted in the expression of the stable DNA replication phenotype in the absence of the *oriC* site or an intact *dnaA* gene (Figure 4) thus appear to have activated an alternative pathway for initiation of chromosome replication independent of these two normally required functions. The *sdrA* initiation system allows for chromosome replication sufficient for growth of the cells. Yet, it should be noted that the *sdrA ΔoriC* and *sdrA dnaA::Tn10* strains exhibited sensitivity to rich media and abnormal cell-size distribution (unpublished results) suggesting that the coordination between chromosome replication and cell growth and division was severely perturbed.

It is at present not clear whether the *sdrA* initiation pathway represents a truly alternative initiation system for chromosome replication, i.e., as a natural *oriC-dnaA* initiation 'bypass' which under normal conditions is turned off, for example, by the product of the *sdrA* gene as earlier suggested (Kogoma *et al.*, 1981; Torrey and Kogoma, 1982), or whether it reflects the activation of an integrated replicon such as a plasmid insert or a prophage. The *rac* prophage from which the origin of replication was cloned as a functional origin *(oriJ)* might be regarded as such an example: its origin does not appear to be used *in situ* (Diaz and Pritchard, 1978). Determination of the site(s) of initiation of replication

in the *sdrA* Δ*oriC* strains and subsequent physical analysis should elucidate the mechanism.

In many *E. coli* strains stable DNA replication can be induced by a variety of treatments which are known to activate the *recA*$^+$ *lexA*$^+$-regulated SOS functions (Kogoma *et al.*, 1979). Thus, one can argue that there is an ubiquitous initiation system which might be rendered constitutive by the *sdrA* mutations. The observation that induced stable DNA replication in several *dnaA* mutants is temperature-resistant (Kogoma and Lark, 1975; Ciesla and Jonczyk, 1980; Lark *et al.*, 1981) supports this argument. Apparent coincidence of the genetic map position of the *srdA* mutations with some of the *dasF* mutations selected as suppressors of the temperature- sensitive phenotype of *dnaA* mutations (Atlung, 1981) also points to such a possibility.

A point of discrepancy needs comment: the stable DNA replication in *sdrA2* mutants was previously reported to be dependent on the *dna* protein since it had not been detected at 40°C in the *sdrA2 dnaA5* double mutants in the presence of CM (Kogoma, 1978), whereas the stable DNA replication phenotype was exhibited at 42°C in the *sdrA224 dnaA46* strain (Figure 1). Further detailed study has indicated, however, that if the temperature was raised sometime after the addition of CM, the stable DNA replication in the *sdrA2 dnaA5* mutant became resistant to high temperatures (unpublished data). It appears that in this particular strain the switch from the *oriC*$^+$ *dnaA*$^+$-dependent mechanism to the *sdrA* initiation system occurred gradually. The difference may be due to the different *sdrA* alleles used *(sdrA2 versus sdrA224)* and/or the strains used (15T$^-$ *versus* K-12).

The results presented here also allow us to conclude that the *dnaA* gene product is only essential for initiation of chromosome replication at *oriC*: it has no other essential function in DNA replication (Kogoma and Lark, 1975; Walker *et al.*, 1981) or any other cellular process. On the other hand, it is surprising that the *dnaA* gene could be inactivated by insertion of Tn*10* without deleterious effects on the expression of the co-transcribed and downstream-located *dnaN* gene coding for the essential β subunit of DNA polymerase III (Burgers *et al.*, 1981). Since the *dnaA* gene could be disrupted in *sdrA* strains (this work) and integratively suppressed strains (unpublished results), levels of the *dnaN* gene product sufficient for replication of the chromosome must be produced in these strains. Considerable expression, albeit at a reduced level, of the *dnaN* gene product has been observed from phage λ*tna*406::Tn*10*-2420 (unpublished results). This expression may come from the weak promoter located in front of the *dnaN* gene described by Sakakibara *et al.* (1981), possibly 'helped' by transcription out of the IS*10* sequence of Tn*10* inserted in the *dnaA* gene (Halling *et al.*, 1982).

Materials and methods

Bacterial strains and plasmids

Bacterial strains used are listed in Table I. The *sdrA224* mutation used in this study was isolated in an *E. coli* K-12 strain by T.A.Torrey and introduced into AQ685 by P1-mediated transduction, resulting in AQ694 and AQ699. The phenotype of the new *sdrA* mutation was similar to those of other *sdrA* mutations described previously (Kogoma, 1978; Kogoma *et al.*, 1981). Details of isolation, characterization and genetic mapping of the *sdrA224* mutation will be described elsewhere. The plasmids pFHC270 (from F.Hansen; Hansen *et al.*, 1981) and pOMC14 (from B.Jørgensen and O.Michelsen) are pBR322 derivatives which carry a *Hind*III fragment and a *Bam*HI-*Eco*RI fragment, respectively of the *oriC* region of the *E. coli* chromosome, (see Figure 4C). pJC605 (from W.Ream and A.J.Clark) is also a pBR322 derivative which contains a 3.6-kb *Eco*RI fragment of the *dnaA* region of the *E. coli* chromosome (see Figure 2).

Growth media and growth conditions

These were as described previously (Kogoma, 1978).

Bacteriophages and transduction

λ-Mediated transduction was described earlier (Hansen and von Meyenburg, 1979). The Tn*10* carrying λ*tna*406 phages were isolated in the same way as the earlier described λ*asn*20::Tn*10* (von Meyenburg and Hansen, 1980). The position and orientation of the Tn*10* inserts were determined from restriction analysis. Analysis of the proteins synthesized from the phage DNA after infection of u.v.-killed cells (Hansen and von Meyenburg, 1979) revealed, in accordance with the physical mapping of Tn*10* in λ*tna*406::Tn*10*-2420 (Figure 4), that this phage did not synthesize the 54-K *dnaA* protein as opposed to λ*tna*406 (Hansen and von Meyenburg, 1979) or λ*tna*406::Tn*10*-2123 (Figure 4) which, on the other hand, did not synthesize the *tnaA* gene product (data not shown). The two phages therefore carry the *dnaA* and *tnaA* mutant alleles designated as *dnaA850::*Tn*10* and *tnaA600::*Tn*10*, respectively. The *oriC* deletion phage λ*asn*132 *oriC del-1071* was previously described (von Meyenburg and Hansen, 1980).

Preparation of DBM paper and electrophoretic transfer of DNA

Preparation of DBM paper and transfer of DNA fragments from agarose gel to DBM paper was according to Alwine *et al.* (1979) and Stellwag and Dahlberg (1980). N-(3-nitrobenzyloxymethyl)-pyridinium chloride was from BDH Chemicals Ltd., Poole, UK.

DNA-DNA hybridization

This was performed according to Wahl *et al.* (1979). ^{32}P-labelled probes were prepared by nick-translation as described by Rigby *et al.* (1977).

Other methods

Measurement of DNA synthesis (Kogoma, 1978) and recombinant DNA technology (Timmis *et al.*, 1978) were described earlier.

Acknowledgements

We thank Tove Atlung, Flemming Hansen, Ole Michelsen, Birgitte Jørgensen and Andrew Wright for bacterial strains and plasmids; Luud Koppes and Egon Hansen for advice on nick-translation and preparation of DBM-DNA paper, Flemming Hansen for stimulating discussions, and Ted Torrey for suggestions on improving the manuscript. The work was supported in part by grants from the Danish Natural Science Research Council and the NOVO Foundation to K.v.M. and in part by grants from the National Institutes of Health (GM22092 and MBS Grant PRO 8139) to T.K.

References

Alwine,J.C., Kemp,D.J., Parker,B.A., Reiser,J., Renart,J., Stark,G.R. and Wahl,G.M. (1979) *Methods Enzymol.*, **68**, 220-236.

Atlung,T. (1981) in Ray,D.S. (ed.), *The Initiation of DNA Replication, ICN-UCLA Symposia on Molecular and Cellular Biology,* Vol. **22**, Academic Press, NY, pp. 297-314.

Bagdasarian,M.M., Izakowska,M. and Bagdasarian,M. (1977) *J. Bacteriol.*, **130**, 577-582.

Burgers,P.M., Kornberg,A. and Sakakibara,Y. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 5391-5395.

Ciesla,Z. and Jonczyk,P. (1980) *Mol. Gen. Genet.*, **180**, 617-620.

Diaz,R. and Pritchard,R.H. (1978) *Nature,* **275**, 561-564.

Felton,J., Michaelis,S. and Wright,A. (1980) *J. Bacteriol.*, **142**, 221-228.

Halling,S.M., Simons,R.W., Way,J.C., Walsh,R.B. and Kleckner,N. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 2608-2612.

Hansen,F.G. and von Meyenburg,K. (1979) *Mol. Gen. Genet.*, **175**, 135-144.

Hansen,F.G., Koefoed,S., von Meyenburg,K and Atlung,T. (1981) in Ray, D.S. (ed.), *The Initiation of DNA Replication, ICN-UCLA Symposia on Molecular and Cellular Biology,* Vol. **22**, Academic Press, NY, pp. 37-55.

Hansen,F.G., Hansen,E.B. and Atlung,T. (1982a) *EMBO J.*, **1**, 1043-1048.

Hansen,E.B., Hansen,F.G. and von Meyenburg,K. (1982b) *Nucleic Acids Res.*, **10**, 7373-7385.

Jorgensen,R.A. and Reznikoff,W.A. (1979) *J. Bacteriol.*, **138**, 705-714.

Kogoma,T. (1978) *J. Mol. Biol.*, **121**, 55-69.

Kogoma,T. and Lark,K.G. (1975) *J. Mol. Biol.*, **94**, 243-256.

Kogoma,T., Torrey,T.A. and Connaughton,M.J. (1979) *Mol. Gen. Genet.*, **176**, 1-9.

Kogoma,T., Torrey,T.A., Subia,N.L., and Pickett,G.G. (1981) in Ray,D.S. (ed.), *The Initiation of DNA Replication, ICN-UCLA Symposia on Molecular and Cellular Biology,* Vol. **22**, Academic Press, NY, pp. 361-374.

Kornberg,A. (1980) *DNA Replication*, published by W.H.Freeman and Company, San Francisco.

Lark,K.G., Lark,C.A. and Meenen,E.A. (1981) in Ray,D.S. (ed.), *The Initiation of DNA Replication, ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. **22**, Academic Press, NY, pp. 337-360.

Meijer,M., Beck,E., Hansen,F.G., Bergmans,H.E.N., Messer,W., von Meyenburg,K. and Schaller,H. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 580-584.

Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.

Sakakibara,Y., Tsukano,H. and Sako,T. (1981) *Gene*, **13**, 47-55.

Schaus,N., O'Day,K., Peters,W. and Wright,A. (1981) *J. Bacteriol.*, **145**, 904-913.

Stellwag,E.J. and Dahlberg,A.E. (1980) *Nucleic Acids Res.*, **8**, 299-317.

Sugimoto,K., Oka,A., Sugisaki,H., Takanami,M., Nishimura,A., Yasuda, Y. and Hirota,Y. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 575-579.

Timmis,K., Cabello,F. and Cohen,S.N. (1978) *Mol. Gen. Genet.*, **162**, 121-137.

Torrey,T.A. and Kogoma,T. (1982) *Mol. Gen. Genet.*, **187**, 225-230.

von Meyenburg,K. and Hansen,F.G. (1980) in Alberts,B. and Fox,C.F. (eds.), *Mechanistic Studies of DNA Replication and Genetic Recombination, ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. **21**, Academic Press, NY, pp. 137-159.

von Meyenburg,K., Hansen,F.G., Riise,E., Bergmans,H.E.N., Meijer,M. and Messer,W. (1979) *Cold Spring Harbor Symp. Quant. Biol.*, **43**, 121-128.

von Meyenburg,K., Jørgensen,B.B., Nielsen,J. and Hansen,F.G. (1982) *Mol. Gen. Genet.*, **188**, 240-248.

Wahl,G.M., Stern,M. and Stark,G.R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3683-3687.

Walker,J.R., Haldenwang,W.G., Ramsey,J.A. and Blinkowa,A. (1981) in Ray,D.S. (ed.), *The Initiation of DNA Replication, ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. **22**, Academic Press, NY, pp. 325-336.

Yuasa,S. and Sakakibara,Y. (1980) *Mol. Gen. Genet.*, **180**, 267-273.