Proc. Natl. Acad. Sci. USA
Vol. 76, No. 7, pp. 3097–3101, July 1979
Biochemistry

Isolation and mapping of plasmids containing the *Salmonella typhimurium* origin of DNA replication

(*Salmonella typhimurium* chromosome/DNA initiation/DNA cloning/restriction mapping/plasmid instability)

JUDITH W. ZYSKIND, L. THOMAS DEEN, AND DOUGLAS W. SMITH

Department of Biology, University of California at San Diego, La Jolla, California 92093

Communicated by Dan L. Lindsley, March 22, 1979

ABSTRACT **A purified *Eco*RI restriction endonuclease fragment that determines resistance to kanamycin and is incapable of self-replication was used to select autonomously replicating fragments from an *Eco*RI digest of a *Salmonella typhimurium* F′ plasmid containing the chromosomal region believed to include the *S. typhimurium* origin of DNA replication. Both the F factor and *S. typhimurium* chromosome replication origins were cloned by this procedure. The *Eco*RI fragment containing the *S. typhimurium* origin of replication is 19.4 kilobase pairs long and includes functional *asn*$^+$ and *uncB*$^+$ genes. Restriction endonuclease analysis of deletions obtained from the *S. typhimurium* origin plasmid indicated that the replication origin (*ori* region) is contained within a 3.3-kilobase pair region. Comparison with *Escherichia coli* origin plasmids shows colinearity of gene arrangement on the chromosomes in this region and suggests that some, but not all, regions of the nucleotide sequence in the origin region may be conserved (identical) in these two bacterial species.**

Replication of the *Salmonella typhimurium* and *Escherichia coli* chromosomes, each a single DNA molecule, is initiated at a unique heritable origin and proceeds bidirectionally (1, 2). Recently, plasmids have been isolated that contain the *E. coli* replication origin, *ori* (3–6). Analysis of these *E. coli* origin plasmids has located *ori* to a 422 base pair region of the chromosome between the genes *uncB* and *asn*, at 82 or 83 min on the *E. coli* genetic map (3, 5, 6). The nucleotide sequence of this region has been determined independently by two groups (5, 6), with complete agreement.

Although their genetic maps are very similar (7), *S. typhimurium* and *E. coli* are not closely related members of the family Enterobacteriaceae (8). Because of the divergence of these two species, studies of the pattern of conserved and nonconserved regions of DNA sequences, for example, in the *trp* operon (9), have yielded sequence requirements necessary for regulatory gene activities. Similar comparative studies using the *ori* regions of the *E. coli* and *S. typhimurium* chromosomes should be equally valuable in understanding the initiation process in DNA replication. Here we report the cloning and initial restriction mapping of the *S. typhimurium ori* region by using a newly constructed *S. typhimurium* F′ plasmid as source of the *S. typhimurium* origin and a nonreplicating *Eco*RI fragment containing a gene conferring resistance to kanamycin (Km) for plasmid selection.

MATERIALS AND METHODS

Bacterial Strains, Media, and Genetic Procedures. The F′ containing the *S. typhimurium* replication origin was constructed from the *S. typhimurium* strain SA970 HfrK11 *serA13 rfa-3058* (from K. Sanderson). All other strains were *E. coli* K-12 derivatives. *E. coli* transformation (10), using SK2267 *gal thi endA sbcB15 hsdR4 recA1* [from S. Kushner (11)], phage P1 transduction (12), and conjugation (12), were as described. Selection procedures were as described, using the indicated recipient strains: ER (13) for *asn*$^+$; AB2070 (14) for *ilvE*$^+$, *metE*$^+$, and *mtl*$^+$; AN120 (15) for *uncA*$^+$, *mtl*$^+$, and *xyl*$^+$; AN382 (15) for *uncB*$^+$, *mtl*$^+$, and *xyl*$^+$; KL141 (16) for *pyrE*$^+$, *thyA*$^+$, and *rbs*$^+$; JM15 (17) for *cysE*$^+$; PC236 (12) for *dnaA*$^+$; and TK1068 (18) for *trkD*$^+$ and *ilvD*$^+$. To select for antibiotic resistance, 50 μg of Km (Calbiochem) per ml, 10 units of ampicillin (Calbiochem) per ml, or 15 μg of tetracycline (Calbiochem) per ml was added to growth medium. Growth media used were M9 (19), antibiotic medium 3 (Difco Penassay broth), and L broth (12).

Plasmids. Plasmids used are listed in Table 1. To assay plasmid loss, exponentially growing cells were transferred from antibiotic-containing Penassay broth to antibiotic-free broth and grown further. Aliquots were then periodically diluted and plated on agar containing and lacking the antibiotic. Plasmid loss is the ratio of antibiotic-resistant colonies to antibiotic-sensitive colonies. Colonies from antibiotic-free plates were also replica-plated to antibiotic-containing plates, yielding identical results.

DNA Isolation. ColE1-derived plasmids (pML21 and pMK2004) were isolated from chloramphenicol-amplified cells (20) by using a cleared lysate procedure, and other plasmids were isolated by the procedure of Currier and Nester (21). F factor was isolated from *E. coli* strain W1485E.

Enzymes and Assay Conditions. Digestion conditions for restriction endonucleases *Bam*HI, *Xho* I, *Hin*dIII, *Pst* I, *Sal* I, and *Bgl* II (from Bethesda Research Laboratories, Rockville, MD) were as recommended by Bethesda Research Laboratories, and those for *Eco*RI [purified through the phosphocellulose step as described (22)] were as described (23). Enough enzyme was used to give complete digestion after 1 hr at 37°C (0.5–2 μg of DNA in 50 μl), followed by enzyme heat inactivation (70°C, 10 min). Ligation reaction mixtures (24) contained 0.5 unit of phage T4 DNA ligase (Miles) and 2–8 μg of DNA in 100 μl.

Gel Electrophoresis. The molecular weights of DNA fragments greater than 10^6 were determined with agarose gels, using *Eco*RI-digested phage λ DNA and F factor DNA as standards. Either a vertical slab gel [0.2 cm × 14 cm × 22 cm; 1% agarose (SeaKem) in Tris/acetate buffer (23); 15 hr, 23°C, 50 V] or the horizontal gel apparatus (200-ml 1% agarose gel bed in Tris/acetate buffer; 24 hr, 4°C, 70 V) previously described (25) was used. The molecular weights of smaller DNA fragments were determined by using electrophoresis (4 hr, 23°C, 200 V) in a vertical slab gel of 7% acrylamide in TBE buffer (26), using *Hin*dIII-digested plasmid R6K as standard (27). DNA bands were visualized by immersing the gel in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MDal, megadalton; kbp, kilobase pairs; Km, kanamycin.

Table 1. Plasmids

Plasmid	$M_r \times 10^{-6}$	Replication origin	Known genes	Copy no.	Plasmid loss
pML21	6.7	ColE1	*kan*	ND	ND
pML31	10.5	F factor	*kan*	ND	ND
pMK2004	3.2	ColE1	*amp tet kan*	ND	ND
pOC15	5.7	*E. coli*	*asn amp*	4.2	2.0
pJZ1	16.5	*S. typhimurium*	*asn uncB kan*	0.8	2.0
pJZ5	14.7	*S. typhimurium*	*asn uncB kan*	0.9	2.0
pJZ7	7.2	*S. typhimurium*	*asn uncB kan*	5.5	4.2
pJZ9	11.6	*S. typhimurium*	*asn kan*	7.3	8.2
pJZ15	8.1	*S. typhimurium*	*asn kan*	9.0	9.4
pJZ2	10.5	F factor	*kan*	ND	>26

Molecular weights (M_r) for the pJZ plasmids are a summation of restriction fragment molecular weights. F factor genes are not listed. Copy number is the number of plasmid molecules per chromosomal equivalent. Plasmid loss is given as the number of generations for 50% of the cells to lose the plasmid when grown in the absence of the antibiotic. ND, not determined.

running buffer (Tris/acetate) containing 0.5 μg of ethidium bromide per ml for 20 min, followed by UV illumination.

Purification of *kan*-Fragment. *Eco*RI digestion of plasmid pML21 yields two fragments, of molecular weights 4.5×10^6 and 2.2×10^6 (24). The larger fragment (*kan*-fragment) confers resistance to Km and cannot self-replicate; the smaller fragment (mini-ColE1) contains the ColE1 replication origin. The *kan*-fragment, isolated as described (24), contained no mini-ColE1 fragment detectable by electrophoresis, and no Km-resistant colonies resulted from transformation of any *E. coli* strain with this preparation.

Determination of Copy Number. Each plasmid tested was used to transform *E. coli* DF1323 *thr leu* Δ(*trpE5*) *thy recA* (from D. Figurski). Cells were grown for four to five generations to 2×10^8 cells per ml in supplemented M9 medium containing [^{3}H]thymine [2 μg and 2 μCi per ml (1 Ci = 3.7×10^{10} becquerels)], Sarkosyl lysates were prepared, plasmid and chromosomal DNA were separated in propidium diiodide/CsCl gradients (48 hr, 36,000 rpm, 15°C), fractions were collected, and radioactivity was assayed as described (12). Copy number was determined from the radioactivity in the two DNA bands, and from the molecular weights of the plasmids and the chromosome (2500×10^6). Because only supercoiled plasmid DNA is assayed by this procedure, a lower limit for the copy number is obtained.

Containment. These experiments were conducted with EK1 and P2 containment.

RESULTS

F′ Plasmids Containing the *S. typhimurium* DNA Replication Origin. The *S. typhimurium* replication origin, located between *cysG* (72 min) and *ilv* (83 min), probably lies between *cysE* (79 min) and *ilv* (2). An F′ plasmid containing the region of the *S. typhimurium* chromosome between *cysE* and *ilv* was isolated by using *E. coli* AI214 as recipient and *S. typhimurium* SA970 HfrK11 as donor and selecting *pyrE*$^+$, *mtl*$^-$, *xyl*$^-$ clones. [*E. coli* AI214 (constructed by A. Iriye) is a r_K^-, *thr*$^+$ and *tnT300*, *recA56* transductant of *E. coli* AT2538 *thi pyrE argE his proA thr leu mtl xyl strA*, constructed by using KH802 r_K^- m_K^+ *met* (from M. Kahn) and JC10240 HfrP045 *srl300::tnT300 thr ilv spc recA56 thi* (from L. Csonka) as donors and selecting for threonine-independent tetracycline-resistant clones that were recombination deficient in conjugation.] One clone (TD27) transfers *cysE*, *pyrE*, *dnaA*, *uncA*, *uncB*, *asn*, *trkD*, *rib*, and *ilvE*, but not *ilvD*, *metE*, *argH*, *xyl*, or *mtl*, and is sensitive to the male-specific RNA phage f2. *E. coli* AI214 is f2-resistant, does not transfer the above markers, and contains no isolatable supercoiled DNA species. All F factor *Eco*RI fragments, in addition to others, are present in *Eco*R1-digested supercoiled DNA isolated from TD27 (Fig. 1). This F′ plasmid, called FST27, has an approximate molecular weight of 156×10^6.

Faster-growing derivative clones of TD27 spontaneously arise during growth. These clones contain F′ plasmids in which part of the original F′ prime, FST27, has been spontaneously deleted. For example, all F factor *Eco*RI fragments are present, but the second-largest *Eco*RI fragment of FST27 and four other smaller fragments are absent (Fig. 1) in *Eco*RI-digested supercoiled DNA isolated from one such clone (DS705). This new F′, called FST27-D1, transfers *pyrE* and *ilvE*, but not *uncA*, *uncB*, *asn*, or *trkD*, indicating that these four genes have been deleted from FST27.

Chimera Plasmids Containing Replication Origins from F′ FST27. The highly transformable plasmid-free *E. coli* strain SK2267 (11) was transformed with *Eco*RI-digested FST27 DNA ligated to *kan*-fragment, and Km resistance was selected. Two types of plasmids were present in the 36 Km-resistant clones obtained. One plasmid type, present in 32 isolates and typified by plasmid pJZ2, was identical to pML31, the mini F-*kan* plasmid (24). Both pJZ2 and pML31 (Fig. 1) contain *kan*-fragment and F factor *Eco*R1 fragment 5, the fragment that contains the F factor vegetative replication origin (24, 28). The other plasmid type, found in four isolates and typified by plasmid pJZ1, contains two *Eco*RI fragments, *kan*-fragment and a 12-megadalton (12.0-MDal) fragment (Fig. 1). This 12-MDal fragment migrates identically with the second largest FST27 *Eco*RI fragment, one of the fragments absent in the deletion F′ FST27-D1 (Fig. 1). Transformation showed that

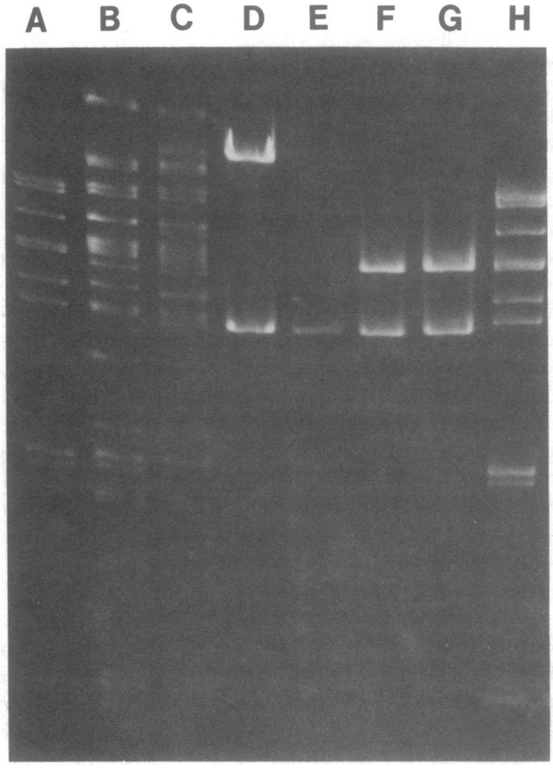

FIG. 1. Horizontal agarose gel electrophoresis of *Eco*RI-digested DNA species. Lane A, F factor. Fragment sizes: 14.4, 14.2, 11.5, 9.9, 9.5, 8.3, 7.8, 4.8 (doublet), 4.5, and 2.4 (doublet) kilobase pairs (kbp). Smaller fragments (28) are not visible. B, FST27-D1; C, FST27; D, pJZ1; E, *kan*-fragment; F, pJZ2; G, pML31; H, F factor.

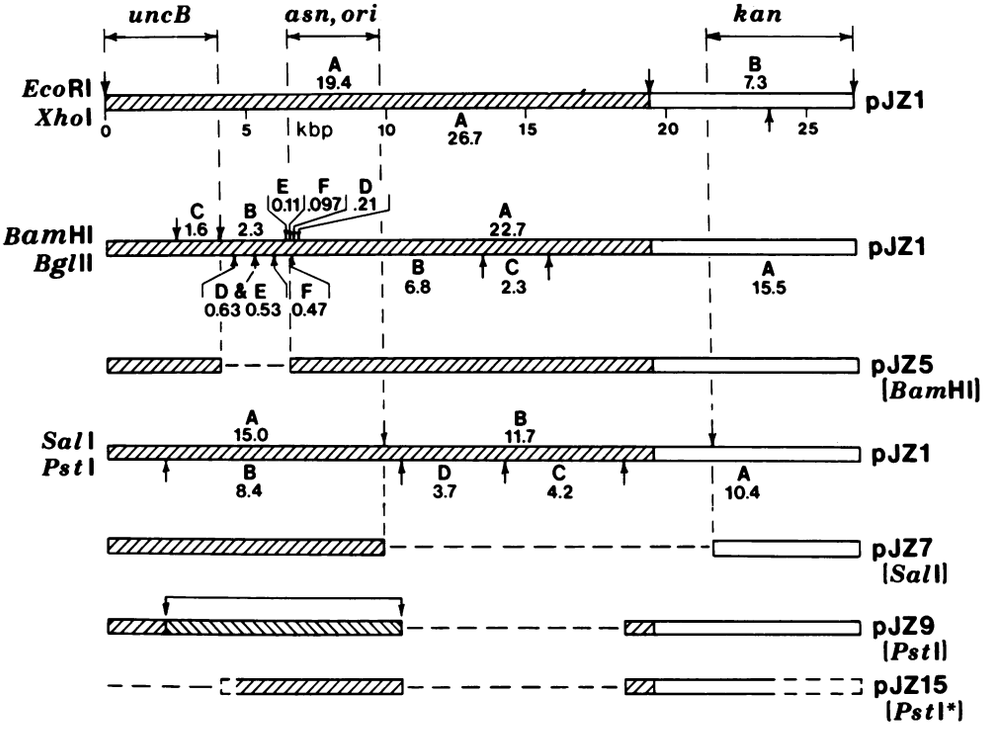

FIG. 2. Restriction endonuclease recognition sites in pJZ1 and deletion derivative plasmids. The restriction endonuclease used to generate each deletion plasmid is given in brackets underneath the respective plasmid name. Hatched regions designate *S. typhimurium* chromosomal DNA, and open regions indicate *kan*-fragment. ↓ ↓ indicates an inverted *Pst* I fragment.
* pJZ15 contains a spontaneous deletion as well as the *Pst* I-derived deletion. The exact ends of the spontaneous deletion are unknown as shown by the dashed bar.

both *asn* and *uncB* genes are present on this plasmid DNA. Thus, the 12-MDal *Eco*RI fragment is identical physically and genetically to a selected portion of the source *S. typhimurium* F′ FST27 DNA. Contour length measurements of open circular pJZ1 DNA visualized using Kleinschmidt electron microscopy (data not shown) gave a molecular weight of 16.2×10^6, demonstrating that pJZ1 does not possess any unusual additional structural features.

Restriction Analysis of pJZ1. Restriction maps of pJZ1 (Fig. 2) were deduced from restriction fragment molecular weights obtained from gel electrophoresis of single and double digests of pJZ1 and deletion derivative plasmids of pJZ1 with *Bam*HI, *Bgl* II, *Eco*RI, *Xho* I, *Pst* I, and *Sal* I endonucleases.

Deletion Derivative Plasmids of pJZ1. Deletion plasmids were isolated from Km-resistant clones of *E. coli* SK2267 transformed with self-ligated *Bam*HI, *Sal* I, or *Pst* I digests of pJZ1; the plasmids were analyzed by gel electrophoresis. Plasmid pJZ5, present in each of two clones from the *Bam*HI digest, is missing pJZ1 *Bam*HI fragments B and E (Figs. 2 and 3), but transformation showed that pJZ5 is still $uncB^+$ and asn^+. Plasmid pJZ7, present in each of four clones from the *Sal* I digest, is missing the pJZ1 *Sal* I fragment B (Figs. 2 and 3), which includes part of the *kan*-fragment not required for Km resistance (29), and is still asn^+ and $uncB^+$.

All plasmids found in nine clones from the *Pst* I digest are asn^+ but $uncB^-$, and only one has *Pst* I fragments found in pJZ1. This plasmid, pJZ9, contains the pJZ1 *Pst* I fragments A and B (Fig. 3), but the orientation of these two *Pst* I fragments in pJZ9 is the reverse of that in pJZ1, as shown by *Sal* I analysis. The *uncB* gene is presumably inactive due to a *Pst* I site present within it. The other eight *Pst* I deletion plasmids possess only one *Pst* I site and one *Eco*RI site (e.g., Fig. 3). Restriction analysis showed that they have lost pJZ1 *Pst* I fragments C and D (Fig. 2) and have also spontaneously lost part of the *kan*-fragment and part of the *uncB* gene. A restriction map of the smallest of these eight plasmids (pJZ15) is shown in Fig. 2.

Cloning pJZ1 *Pst* I Fragment B by Using Plasmid pMK2004. The plasmid pMK2004 (19) has a single *Pst* I site (Fig. 3), within the *amp* gene. Plasmid pJZ19, consisting of pMK2004 and pJZ1 *Pst* I fragment B (Fig. 3), was isolated from a tetracycline-resistant, ampicillin-sensitive, Km-resistant clone of *E. coli* D110 *polA1 thy endA* (from C. Richardson) trans-

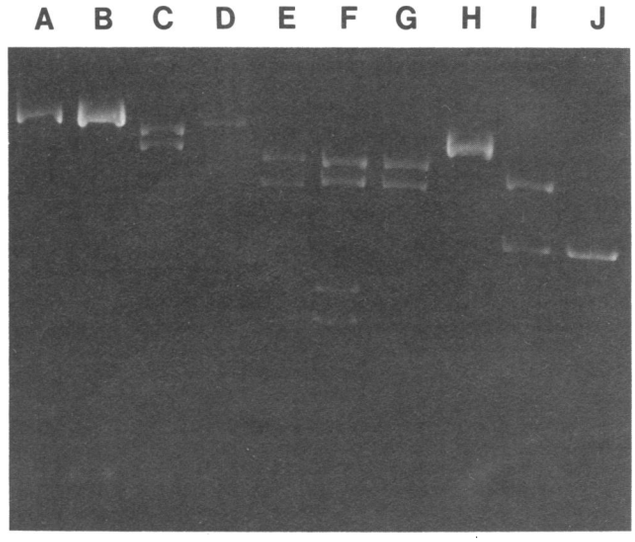

FIG. 3. Vertical agarose gel electrophoresis of restriction endonuclease-digested pJZ1 and deletion derivatives of pJZ1. Lane A, *Bam*HI-digested pJZ1; B, *Bam*HI-digested pJZ5; C, *Sal* I-digested pJZ1; D, *Sal* I-digested pJZ7; E, *Sal* I-digested pJZ9; F, *Pst* I-digested pJZ1; G, *Pst* I-digested pJZ9; H, *Pst* I-digested pJZ15. I, *Pst* I-digested pJZ19, J, *Pst* I-digested pMK2004.

formed with a ligated mixture of *Pst* I-digested pJZ9 and pMK2004. Plasmid pMK2004 has only the ColE1 DNA replication origin, and use of this origin requires a functional *polA* gene (30). Hence, the ability of pJZ19 to replicate in a *polA* mutant indicates that the *S. typhimurium* origin for replication is used and is located within the pJZ1 *Pst* I fragment, and that use of this origin can occur in *polA1* mutants.

Properties of Plasmids Containing a Bacterial Replication Origin. The copy number of pJZ1 and pJZ5 is less than one plasmid molecule per chromosomal equivalent (Table 1). However, deletions either within the *uncB* gene (pJZ9 and pJZ15) or to the right of the *asn* gene (pJZ7) raise the copy number considerably. Plasmid pOC2 (5) consists of the *E. coli* chromosome *Eco*RI fragment containing the *E. coli* replication origin (*ori*) and of an *Eco*RI fragment conferring ampicillin resistance. We found the copy number of pOC15, a *Hin*dIII deletion plasmid of pOC2, to be 4.2, close to the reported value of 2–4 (5).

All bacterial origin plasmids examined are spontaneously lost at an exponential rate during cell growth when selective pressure is removed (Table 1), whereas the F factor origin plasmid pJZ2 is stable. The deletion derivative plasmids of pJZ1 are lost at a lower rate than is pJZ1, and the rate of loss is inversely correlated with the copy number of the plasmid (Table 1). Cultures of cells containing plasmids that are lost at high rates exhibit abnormally long generation times and contain many antibiotic-sensitive cells. For example, only 25% of SK2267 (pJZ1) cells grown in broth containing Km form colonies when plated on agar plates containing Km.

Comigration of Restriction Fragments from *S. typhimurium* and *E. coli* Origin Plasmids. Only the pJZ1 *Bam*HI fragments E and F and the pJZ1 *Bgl* II fragment F comigrated with fragments of *Bam*HI or *Bgl* II digests of pOC15. The smallest *Bgl* II fragment in each plasmid (pJZ1 *Bgl* II fragment F, Fig. 2) contains two *Bam*HI sites in identical positions, and the smallest *Bam*HI fragment in each plasmid (pJZ1 *Bam*HI fragment F, Fig. 2) contains a *Bgl* II site. The restriction maps presented in Fig. 4 of the *ori* regions of *E. coli* (5) and of *S. typhimurium* were oriented by aligning the smallest *Bam*HI fragments in each plasmid.

DISCUSSION

Two different *Eco*RI fragments containing DNA replication origins, from the F factor and the *S. typhimurium* chromosome, were cloned as plasmids from the *S. typhimurium* F′ FST27. A nonreplicating *Eco*RI fragment conferring Km resistance was used for selection. Restriction analysis of the two different types of plasmids isolated in these experiments showed that one of these types—e.g., pJZ2—was identical to pML31 (24), and contained the *Eco*RI fragment 5 from the F factor, the fragment that contains the F factor origin for vegetative replication [*oriV* (28)] in addition to *kan*-fragment. The other type contained two *Eco*RI fragments, the *kan*-fragment and a fragment identical in size to *Eco*RI fragment 2 of the F′ FST27. Four independently isolated clones contained this plasmid type—e.g., pJZ1—with no detectable differences in restriction fragment molecular weights or in genetic or physiological properties. The evidence that the *Eco*RI fragment isolated in the plasmid pJZ1 contains the *S. typhimurium* origin and does not contain any *E. coli* or F factor DNA is as follows. (*i*) The 4.5-MDal *kan*-fragment does not contain an origin for DNA replication (ref. 24; data not shown). (*ii*) The 12.0-MDal *Eco*RI fragment in pJZ1 comigrates in agarose gels with the second-largest *Eco*RI fragment of the source *S. typhimurium* F′ plasmid, FST27, and this fragment is not present in F factor DNA (Fig. 1). The F factor origin is contained on an *Eco*RI fragment 6.0 MDal in size (24) that is not present in pJZ1. (*iii*) The plasmid pJZ1 carries functional *uncB*$^+$ and *asn*$^+$ genes. These genes bracket the *E. coli* replication origin. (*iv*) The *E. coli Eco*RI fragment containing the replication origin is 5.9 MDal and does not contain a functional *uncB*$^+$ gene. Further, many restriction sites are dissimilar (Fig. 4) between pJZ1 and the *E. coli* origin region. These facts argue that pJZ1 was not derived from the *E. coli* chromosome or from F factor.

As has been observed for *E. coli* origin plasmids (5, 6), *S. typhimurium* origin plasmids (pJZ1 and its derivatives) are not stably maintained in the absence of selective pressure (Table 1). In contrast, F factor origin plasmids—e.g., pJZ2—appear stable. Apparently, incompatibility of pJZ1 and its derivatives with the *E. coli* chromosome decreases the stability of these plasmids. However, deletions in pJZ1, either to the left or the right of the replication origin, increase the stability of the re-

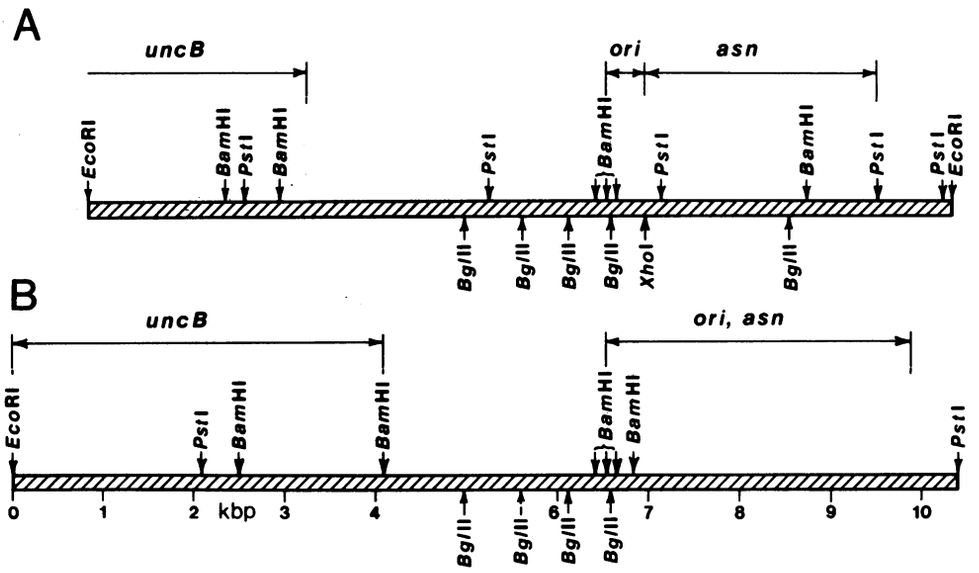

FIG. 4. Restriction endonuclease recognition sites in the *ori* region of the *E. coli* (*A*) and *S. typhimurium* (*B*) chromosomes.

sulting plasmids (Table 1). The increased stability associated with decreased plasmid size may be the result of loss of genes whose products are detrimental to cell growth when present in higher than normal amounts. Supporting this possibility is the observation that pJZ9, which contains an inverted pJZ1 *Pst* I fragment B, is more stable than pJZ7, even though all of pJZ9 is contained within pJZ7. This suggests that a disruption of the nucleotide sequence at the *Pst* I site in the *uncB* gene, a site outside the chromosome origin region, increases plasmid stability by preventing the production of a functional *uncB* gene product. However, all derivative plasmids obtained via deletions of the original *S. typhimurium* and *E. coli* origin plasmids remain unstable to some extent (refs. 5 and 6; Table 1). Thus, incompatibility of these plasmids with the *E. coli* chromosome also appears to affect plasmid stability.

A comparison of the *E. coli* origin plasmid pOC15 with the *S. typhimurium* origin plasmid pJZ1 shows both similar and different features. Genetically, the order of the genes *uncB* and *asn*, their physical distance from each other, and their position relative to the *ori* region are very similar (Fig. 4). The relative positions of some of the restriction sites are similar and others are different. Apparent conservation of restriction sites is most notable within the *ori* region and just to the left of this region (Fig. 4). In particular, the positions of the *Bgl* II and *Bam*HI sites within, and defining, the pJZ1 *Bgl* II fragment F and *Bam*HI fragments E and F appear to be identical in the two plasmids. Nearly all other restriction endonuclease sites examined not mentioned above are different in the two plasmids. In particular, (*i*) differences exist within the regions containing the *asn* and *uncB* genes; (*ii*) the *Eco*RI sites are both different, relative to the common *Bgl* II and *Bam*HI sites; and (*iii*) the extent of the *ori* region in pOC15 is defined by an *Xho* I site that is missing in pJZ1, and conversely pJZ1 possesses a *Bam*HI site in this region not found in pOC15. Thus, the nucleotide sequence in the *ori* region of *S. typhimurium* differs in part from that of *E. coli*. Nevertheless, the sequences are sufficiently similar that the *S. typhimurium* origin plasmid can adequately utilize the *E. coli* DNA replication apparatus for its replication. A detailed comparison of the nucleotide sequence of the origin region of these two bacterial chromosomes should prove useful in understanding which nucleotides within this region are essential for the initiation process.

Note Added in Proof. Plasmid pMK2004 has a single *Bam*HI site within the *tet* gene. Plasmids constructed by insertion of pJZ1 *Bam*HI fragments D and F (Fig. 2) into the pMK2004 *Bam*HI site can replicate in *E. coli* D110 *polA1* and are Km-resistant, ampicillin-resistant, tetracycline-sensitive, and asparagine-negative. Thus, the gene sequence *uncB-ori-asn* is identical in *E. coli* and in *S. typhimurium*, and the *S. typhimurium ori* region is localized to 317 base pairs.

We thank M. Kahn and S. Gelvin for many helpful suggestions; B. Bachmann, P. Carl, L. Csonka, W. Epstein, D. Figurski, D. Helinski, M. Kahn, S. Kushner, W. Messer, and C. Richardson for bacterial strains; and W. Messer and Y. Hirota for providing us with manuscripts prior to publication. This work was supported by a National Institutes of Health Postdoctoral Fellowship (GM05459) to J.W.Z., and by grants from the National Institutes of Health (GM21978) and from the Cancer Research Coordinating Committee, University of California.

1. Hohlfeld, R., & Vielmetter, W. (1973) *Nature (London) New Biol.* **242,** 130–132.
2. Fujisawa, T. & Eisenstark, A. (1973) *J. Bacteriol.* **115,** 168–176.
3. Yasuda, S. & Hirota, Y. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5458–5462.
4. Diaz, R. & Pritchard, R. H. (1978) *Nature (London)* **275,** 561–564.
5. Meijer, M., Beck, E., Hansen, F. G., Bergmans, H. E. N., Messer, W., von Meyenburg, K. & Schaller, H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 580–584.
6. Sugimoto, K., Oka, A., Sugisaki, H., Takanami, M., Nishimura, A., Yasuda, S. & Hirota, Y. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 575–579.
7. Sanderson, K. E. & Hartman, P. E. (1978) *Microbiol. Rev.* **42,** 471–519.
8. Sanderson, K. E. (1976) *Annu. Rev. Microbiol.* **30,** 327–349.
9. Lee, F., Bertrand, K., Bennett, G. & Yanofsky, C. (1978) *J. Mol. Biol.* **121,** 193–217.
10. Cohen, S. N., Chang, A. C. Y. & Hsu, L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 2110–2114.
11. Kushner, S. R. (1978) in *Genetic Engineering,* eds. Boyer, H. & Nicosia, S. (Elsevier/North-Holland, Amsterdam), pp. 17–23.
12. Zyskind, J. W. & Smith, D. W. (1977) *J. Bacteriol.* **129,** 1476–1486.
13. Cedar, H. & Schwartz, J. H. (1969) *J. Biol. Chem.* **244,** 4112–4121.
14. Wechsler, J. A. & Adelberg, E. A. (1969) *J. Bacteriol.* **98,** 1179–1194.
15. Butlin, J. D., Cox, G. B. & Gibson, F. (1973) *Biochim. Biophys. Acta* **292,** 366–375.
16. Low, B. (1973) *J. Bacteriol.* **113,** 798–812.
17. Jones-Mortimer, M. C. (1968) *Biochem. J.* **110,** 589–595.
18. Epstein, W. & Kim, B. S. (1971) *J. Bacteriol.* **108,** 639–644.
19. Kahn, M., Kolter, R., Thomas, C., Figurski, D., Meyer, R., Remault, E. & Helinski, D. (1979) *Methods Enzymol.* **68,** in press.
20. Katz, L., Kingsbury, D. T. & Helinski, D. R. (1973) *J. Bacteriol.* **114,** 577–591.
21. Currier, T. C. & Nester, E. W. (1976) *Anal. Biochem.* **76,** 431–441.
22. Greene, P. J., Betlach, M. C., Boyer, H. W. & Goodman, H. M. (1974) in *Methods in Molecular Biology,* ed. Wickner, R. B. (Dekker, New York), Vol. 7, pp. 87–111.
23. Fijimura, F. K., Zyskind, J. W. & Smith, D. W. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 559–562.
24. Lovett, M. A. & Helinski, D. R. (1976) *J. Bacteriol.* **127,** 982–987.
25. Kaplan, D. A., Russo, R. & Wilcox, G. (1977) *Anal. Biochem.* **78,** 235–243.
26. Maniatis, T., Jeffrey, A. & Van deSande, H. (1975) *Biochemistry* **14,** 3787–3794.
27. Kolter, R. & Helinski, D. R. (1978) *Plasmid* **1,** 571–580.
28. Skurray, R. A., Nagaishi, H. & Clark, A. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 64–68.
29. Armstrong, K. A., Hershfield, V. & Helinski, D. R. (1977) *Science* **196,** 172–174.
30. Kingsbury, D. T. & Helinski, D. R. (1973) *J. Bacteriol.* **114,** 1116–1124.