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Revised Interpretation of the Origin of the pSC101 Plasmid

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Data are presented indicating that the pSC101 plasmid was not derived by recircularization of a mechanically sheared fragment of R6-5 plasmid deoxyribonucleic acid, as was originally believed.

During the past several years, there has been considerable experimental interest in the pSC101 plasmid, which has been used as a cloning vehicle in recombinant deoxyribonucleic acid (DNA) experiments (2, 4), as a model replicon for investigation of plasmid DNA replication (4, 21, 23), and as a recipient or donor genome in studies of translocation of antibiotic resistance determinants (11-13). This plasmid was originally isolated after transformation of *Escherichia coli* with mechanically sheared DNA from the large antibiotic resistance plasmid R6-5 (3) and was believed to have been formed by recircularization of a fragment of R6-5 DNA. We no longer believe this interpretation to be correct. This communication reports our revised view of the origin of the pSC101 plasmid.

R6-5 is a tetracycline (Tc)-sensitive derivative of the R6 plasmid (19, 20). Although R6-5 carries the same Tc resistance gene as R6, expression of this gene in R6-5 is prevented by insertion of a short DNA sequence that is homologous with the IS3 insertion element (17, 19). Two additional copies of the inserted DNA sequence are present in inverted orientation in the vicinity of the Tc resistance gene. In the experiments leading to isolation of the pSC101 plasmid (3), R6-5 plasmid DNA was sheared for varying periods of time, and DNA samples removed from the shearing vessel at different times were studied for their ability to transform *E. coli* for the separate antibiotic resistance determinants carried by R6-5. After extensive shearing of R6-5 DNA, transformants were isolated that expressed Tc resistance but that did not express the other resistance determinants on the R6-5 plasmid.

Although Tc resistance is not expressed by R6-5, excision of the insertion element adjacent to the Tc resistance gene leads to expression of Tc resistance (1, 19). We believed that a replicating R6-5 fragment produced by mechanical shearing and lacking the insertion element copy had recircularized in vitro at a very low frequency, yielding a new plasmid. Such in vitro recircularization of linear DNA fragments has been demonstrated recently for other molecules (5). An alternative possibility was that a new plasmid had been formed from a DNA segment that had been excised spontaneously from the R6-5 plasmid in vivo; formation of such "mini" plasmids by apparent excision of replicating segments of large plasmids has also been described (6, 9, 14).

The plasmid we isolated after transformation of *E. coli* by sheared R6-5 DNA was originally designated TC6-5 (3), and shortly afterwards was renamed pSC101 (4) in accordance with the recommendations of a proposal for a uniform plasmid nomenclature (15). Subsequent findings from our laboratory have raised questions about the adequacy of our earlier interpretation of the origin of the pSC101 plasmid, and these findings are summarized as follows. (i) Whereas the *Eco*RI cleavage site of pSC101 does not interfere with expression of the Tc resistance gene of the plasmid (4), we have repeatedly been unable to clone an *Eco*RI-generated fragment of R6-5 or R6 carrying Tc resistance, suggesting that in these plasmids the *Eco*RI cleavage site may be located within the Tc resistance gene or within an element controlling expression of this gene. (ii) A fragment of R6-5 DNA carrying replication functions has been cloned after *Eco*RI endonuclease digestion of the plasmid, and this fragment maps at a considerable distance from the Tc resistance gene of R6-5 (22). However, multiple origins of replication have been identified on large antibiotic resistance plasmids related to R6-5 (16), and it seemed possible that pSC101 contained an R6-5 replication origin other than the one that had been cloned. (iii) The pSC101 plasmid is entirely compatible with R6-5, whereas plasmids carrying an R6-5 replication system show incompatibility with the parent (1). However, we have recently observed (A.

C. Y. Chang and S. N. Cohen, manuscript in preparation) that separate plasmids containing the same replication origin can exist compatibly within a bacterial cell. (iv) Although DNA-DNA and DNA-ribonucleic acid hybridization experiments on nitrocellulose membranes showed homology between pSC101 and R6-5, only a small fraction of pSC101-specific radioactivity hybridizes with R6-5 (A. C. Y. Chang and S. N. Cohen, unpublished data; S. Falkow, personal communication). The sequences of R6-5 that are homologous with pSC101 do not appear to be located in the vicinity of the Tc resistance gene. (v) We have been unable to demonstrate heteroduplex formation between the two plasmids by electron microscopy (D. J. Kopecko, A. C. Y. Chang, and S. N. Cohen, unpublished data). However, we expected that intrastrand annealing of the inverted repeat sequences of the Tc resistance loop of R6-5 would reduce the frequency of any intermolecular heteroduplex formation between this segment of R6-5 and other plasmids that carry the homologous region. In addition, the large size of the R6-5 plasmid relative to that of pSC101 would be expected to contribute to a low frequency of R6-5/pSC101 heteroduplexes, since homoduplexes between R6-5 strands would be favored.

Because of the findings outlined above, we recently carried out additional experiments intended to determine whether the *Eco*RI, *Hin*dIII, *Bam*I, and *Sal*I restriction endonuclease sites of pSC101, which are known to be in the vicinity of or within the Tc resistance gene of this plasmid, exist in the same spatial relationship in pSC101 and R6-5. These experiments have involved use of the pKT007 plasmid (K. Timmis, F. Cabello, and S. N. Cohen, manuscript in preparation), which contains an *Hin*dIII endonuclease-generated Tc resistance fragment of R6 DNA that has been linked to a ColE1-derived cloning vehicle (i.e., pML21, reference 10).

Plasmid pSC101 contains cleavage sites for the restriction endonucleases *Eco*RI, *Hin*dIII, *Bam*I, and *Sal*I at known distances from each other (2a, 8, 18); moreover, the *Bam*I, *Hin*dIII, and *Sal*I sites are known to be located within the Tc resistance operon of the plasmid. However, we observed that the R6 DNA segment contained in pKT007 lacks a *Sal*I site even though it carries the only Tc resistance gene known to be present on the R6 plasmid. Although the segment of R6 cloned in pKT007 does contain *Eco*RI, *Hin*dIII, and *Bam*I sites (Fig. 1), they are at different distances from each other in pKT007 and pSC101 (Table 1). Moreover, the R6 DNA component of the pKT007 plasmid contains an *Eco*RI cleavage site between one of the *Hin*dIII sites used to join the R6 Tc resistance fragment to pML21 and the single *Bam*I site present on the fragment; in pSC101 the sequence of the three restriction endonuclease cleavage sites is *Eco*RI-*Hin*dIII-*Bam*I.

Although it has been shown that microevolution of plasmid DNA can result in different restriction endonuclease digestion patterns in DNA segments that appear indistinguishable by heteroduplex analysis (S. N. Cohen, F. Cabello, A. C. Y. Chang, and K. Timmis, *in* D. Schlessinger, ed., *Microbiology–1978*, in press), we nevertheless conclude from the various findings summarized here and the data presented in Fig. 1 and Table 1 that our original interpretation of the derivation of the pSC101 plasmid is not correct. Localization of the cloned replication region of R6-5 at a considerable distance from the Tc gene loop (22), the observed compatibility of pSC101 with R6-5 and with plasmids carrying the R6-5 replication origin (1), and restriction enzyme cleavage patterns showing a different spatial relationship of endonuclease cleavage sites in the Tc resistance regions of R6 and pSC101, taken together, provide compelling evidence that the Tc resistance gene and replication region of pSC101 were not derived by recircularization of a linear DNA fragment produced by shearing of the R6-5 plasmid.

Possibly, contamination of transforming R6-5 DNA preparations with DNA from some other naturally occurring Tc resistance plasmid may have led to the isolation of Tc-resistant transformants containing a small replicon that we believed had been formed from sheared R6-5 DNA. Because of the high sensitivity of the transformation assay, as little as 10^{-5} or 10^{-6} μg of contaminating plasmid DNA could have yielded such Tc-resistant colonies. In this regard, it should be pointed out that pSC101 has been found by nitrocellulose membrane hybridization studies (Chang and Cohen, unpublished data) to show extensive nucleotide base sequence homology with the SP219 plasmid (7, 24). In addition, the pSC101 and SP219 plasmids show indistinguishable cleavage patterns for the *Bam*I, *Eco*RI, *Hin*cII, *Hpa*I, *Mbo*II, and *Sma*I restriction endonucleases (P. Meacock and S. N. Cohen, unpublished data). It seems possible that pSC101 may have been derived from contaminating SP219 DNA or, alternatively, that both plasmids may be independent natural isolates that were derived separately by a similar mechanism.

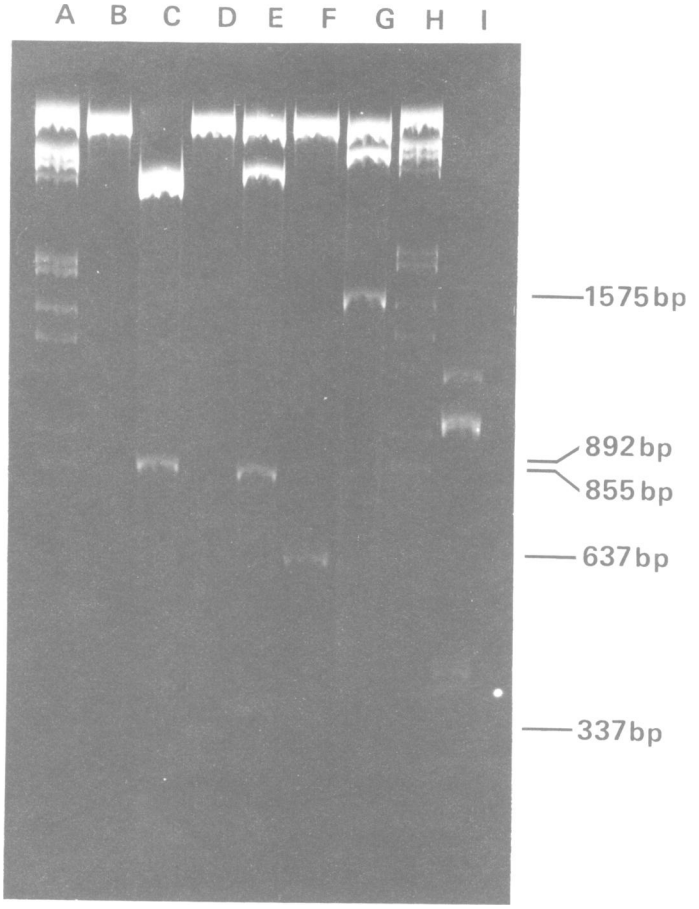

FIG. 1. *Agarose gel electrophoresis of endonuclease-treated plasmid DNA. The experimental procedures for isolation of DNA, endonuclease digestion, and analysis of DNA by electrophoresis in 2% polyamylamide–0.5% agarose gels were described previously (16). Columns (A) and (H) contained a bacteriophage λ DNA molecular weight standard treated with EcoRI and HindIII enzymes; column I contained ColE1 DNA digested with HindIII. The molecular lengths of the λ DNA fragments (kilobase pairs) are 20.7, 5.02, 4.71, 4.06, 1.84, 1.71, 1.48, 1.2, 0.922, 0.877, and 0.508. The sizes of the ColE1 fragments (in kilobase pairs) are: 1.09, 1.05, 0.9, 0.435, 0.422, 0.410, and 0.252. Columns (B), (D), and (F) contain 1.0 μg of pKT007 DNA. DNA samples were treated before electrophoresis as follows: columns (B) and (C), HindIII plus EcoRI; columns (D) and (E), HindIII plus BamI; columns (F) and (G), HindIII plus SalI. The lengths and positions of the relevant fragments of pSC101 and pKT0007 plasmid DNA (Table 1) are indicated.*

TABLE 1. *Restriction nuclease fragments*[a]

Enzyme treatment	Fragment size (base pairs)		
	pKT007	pSC101 (A)	pSC101 (B)
*Hin*dIII + *Eco*RI	892	~50	~50
*Hin*dIII + *Bam*I	855	337	330
*Hin*dIII + *Sal*I	1,575	637	620

[a] The lengths (in base pairs) of restriction-enzyme-generated DNA fragments of pSC101 determined by Hamer and Thomas (8) (A) and by us (B) are shown in comparison with those of fragments generated by treatment of the pKT007 plasmid with the same enzymes. The reproducibility of molecular lengths from the gel mobility of DNA fragments is ±5%.

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