

Plasmid pT181-Linked Suppressors of the *Staphylococcus aureus pcrA3* Chromosomal Mutation

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Received 2 December 1992/Accepted 9 April 1993

Plasmid pT181 replication is affected in hosts carrying the chromosomal *pcrA3* mutation, resulting in significantly lower plasmid copy numbers. Mutations suppressing this effect have been isolated and characterized. The suppressor mutations were found to map in the plasmid *repC* gene and manifested *pcrA* allele specificity, suggesting the existence of a direct RepC-PcrA interaction.

Most plasmids encode only the function(s) required for replication initiation and rely on host functions for other steps of replication. It is conceivable that specific interactions occur during plasmid replication between the plasmid initiator and one or more host replicative enzymes. For most plasmids, our knowledge of such interactions is quite limited.

Plasmid pT181, the prototype of an entire class of plasmids from gram-positive bacteria, replicates by a rolling-circle mechanism (2, 15). The plasmid-encoded RepC protein acts in pT181 replication initiation. The initiation involves nicking of pT181 DNA by RepC at a specific site of the origin (10) and the covalent attachment of RepC to the 5' end of the nick generated (16), while the 3' end is used as the primer for rolling-circle replication.

The isolation of host mutants affecting pT181 replication (7) has led to the identification of the *Staphylococcus aureus* chromosomal gene *pcrA*, whose product is required for pT181 replication. A significant lag in the transition from pT181 replication initiation complexes to productive replication occurs in the *pcrA3* host mutant (8). The PcrA protein was found to have a significant degree of homology with the Rep and UvrD helicases of *Escherichia coli* (5), suggesting that it acts as a helicase in pT181 replication. Though the *pcrA* gene is essential for cell viability (5), the *pcrA3* mutation specifically affects the replication of pT181 and related plasmids and has no effect on the host. In an attempt to understand the basis for this specificity, mutations suppressing the effects of *pcrA3* on pT181 replication have been isolated and characterized.

All pT181 derivatives are maintained at a significantly reduced copy number in the *pcrA3* host mutant SA1560 (7). An increase in this copy number is expected to occur in the presence of mutations suppressing the effect of *pcrA3* on pT181 replication. Since the expression of the pT181 tetracycline (TC) resistance determinant is gene dosage dependent (1), suppressor mutations were isolated by selecting for cells able to grow at higher TC concentrations. To prevent the isolation of pT181 copy number mutants which have the same phenotype, the pT181 derivative pRN8008 (*cop-608*), which has most of the replication control region deleted (1), was used. In a *pcrA3* host, pRN8008 is maintained at a copy number about 20 times lower than in a wild-type host.

Exponentially growing cultures of a *pcrA3* strain harboring pRN8008 were plated on gradient plates in which the

basal layer contained 100 µg of TC per ml and the upper layer was antibiotic free. The few large colonies that developed beyond the zone of confluent growth on these plates were subjected to two single-colony isolations at high TC concentrations (60 to 80 µg/ml), and the plasmid copy numbers were evaluated by gel electrophoresis of whole-cell lysates (12). To ensure the independent isolation of mutants, only one clone having a significantly higher plasmid copy number than the parent was kept from each experiment. Three such clones were selected for further study.

The suppressor mutations of all three clones were found to be plasmid linked. The three pRN8008 derivatives carrying suppressor mutations have been designated pSA3111, pSA3113, and pSA3114. Their copy numbers in the *pcrA3* host SA1560 were significantly higher than that of pRN8008. For pSA3111 and pSA3113, the copy number was almost the same in *pcrA3* (500) and wild-type (800) hosts, while pSA3114 was still maintained at a lower copy number in SA1560 (100) than in the wild-type host SA20 (800) (Fig. 1).

The suppressor mutations were mapped by restriction fragment exchanges among the three mutants and pRN8008 and determination of the copy number of the resulting recombinants in SA1560. All three mutations mapped in the *XbaI-PvuI* fragment (pT181 coordinates 3781 to 4437), which contains most of the *repC* gene (9). The *XbaI-PvuI* fragment from each of the three mutants was cloned into an M13 vector (11), and sequencing was performed by the dideoxynucleotide chain termination method (14) with Sequenase (United States Biochemical) and [³⁵S]dATP. pSA3111 and pSA3113 were found to carry the same change, a C to A in position 4420 of the pT181 sequence, leading to an aspartic acid-to-tyrosine substitution at amino acid residue 57 of the RepC protein. This mutation was designated *repC31*. The *repC32* mutation present in pSA3114 led to a serine-to-isoleucine substitution at amino acid residue 102 of RepC.

The *repC31* allele, which essentially confers on the plasmid a *pcrA3*-insensitive phenotype, has been transferred to the wild-type pT181. The resulting plasmid, pSA3151, was found to be maintained at essentially the same copy number in SA20 and SA1560 (Fig. 1), proving that the effect of the *repC31* mutation not only is expressed in a *cop-608* plasmid, in which RepC synthesis is no longer regulated, but is also expressed in a wild-type background.

Though the *repC31* mutation leads to a change in RepC, it might be argued that it also affects the structure of the plasmid DNA in such a way that its replication becomes more efficient in a *pcrA3* host. To test this possibility, the effect of the RepC31 protein in *trans* on the replication of a

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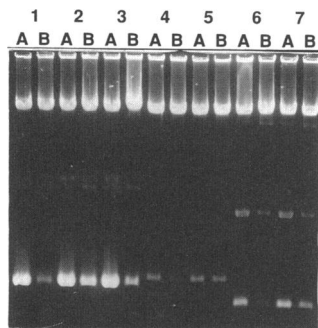


FIG. 1. Agarose gel electrophoresis of whole-cell lysates. Hosts: A, SA20 (*pcrA*⁺); B, SA1560 (*pcrA3*). Plasmids: 1, pRN8008; 2, pSA3111; 3, pSA3114; 4, pT181; 5, pSA3151; 6, pSA7540 and pSA5040; 7, pSA7582 and pSA5040.

plasmid carrying the pT181 origin of replication in a *pcrA3* host was evaluated. The hybrid pSA7540 was used. pSA7540 carries a *repC* gene in which the overlapping origin of replication has been mutationally inactivated without changing the amino acid sequence of RepC (4). As a result, the effect of RepC synthesized by pSA7540 can be tested in *trans* on another pT181 origin without interference by a *cis* origin (4). The *repC31* mutation was introduced into pSA7540 by restriction fragment replacement, generating the hybrid pSA7582. pSA5040 was used as a target plasmid in these experiments. pSA5040 carries a normal pT181 origin, but its *repC* gene has been mutationally inactivated and therefore its maintenance is strictly dependent upon a supply of RepC in *trans* (3). The copy number of pSA5040 in the presence of pSA7540 (*repC*⁺) and pSA7582 (*repC31*) was compared in *pcrA*⁺ and *pcrA3* hosts. The results show that in the presence of RepC31 the copy number of pSA5040 was about the same in SA20 and SA1560, while its copy number was significantly lower in SA1560 in the presence of wild-type RepC (Fig. 1). This result indicates that the effect of the *repC31* mutation on pT181 replication in a *pcrA3* host is due only to the mutation in RepC.

The properties of the plasmids carrying these suppressor mutations suggest either that the mutated RepC can act in pT181 replication in the absence of the PcrA protein or that it enables the plasmid to utilize the mutant PcrA product more efficiently. To differentiate between these possibilities, the mutant plasmids were introduced into another *pcrA* mutant, *pcrA6* (6). While the copy number of most pT181 derivatives is only slightly affected in the *pcrA6* host SA2887 (about 1.5×), the copy number of pRN8008 is reduced about four- to fivefold. The copy number of pSA3111 and pSA3114 in SA2887 was determined and found to be reduced in a similar way to that of pRN8008 (data not shown), showing that the *repC31* and *repC32* mutations cannot suppress the effect of the *pcrA6* mutation. The *pcrA* allele specificity of these *repC* mutations supports a direct interaction between the RepC and PcrA proteins.

A *repC* mutation, *repC35*, which precludes the establishment in a *pcrA3* host of pT181 derivatives carrying it, has been described in a separate study, along with secondary mutations also located in *repC* (*repC41*, *repC44*, and *repC45*) which suppress this effect (6). All the mutations which affect

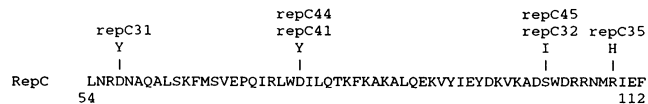


FIG. 2. Region of the RepC protein carrying mutations affecting the plasmid response to the host *pcrA3* mutation.

the plasmid response to the *pcrA3* host mutation are located in the same region of the RepC protein, between amino acid residues 57 and 109 (Fig. 2). This region is highly conserved among plasmids of the pT181 family (13) and has hitherto been mutationally silent. This may define a new domain of the RepC protein, one that interacts with the host PcrA protein.

I thank Ellen Murphy for critical reading of the manuscript.

This work was supported by grant DMB-9004169 from the National Science Foundation.

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