Requirement of the *Escherichia coli* *dnaA* Gene Product for Plasmid F Maintenance

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There are DnaA protein-binding sites in at least one F origin of replication, and only potentially leaky *dnaA*(Ts) mutations had ever been used in previous studies indicating that F replication was independent of the *dnaA* gene product. Here we show that an *Escherichia coli* *dnaA::Tn10* host which does not make a *dnaA* gene product cannot sustain autonomous or integrated F plasmid maintenance.

Integrative suppression is the term used to describe the ability of F and other plasmids to suppress conditional *dnaA*(Ts) mutations when the plasmid becomes integrated into the host chromosome (18, 21). Tresguerres et al. (28) have shown that autonomous F plasmid replication occurs in a *dnaA46*(Ts) host at the restrictive temperature. The traditional interpretation of these results is that F replication does not require the *dnaA* gene product, protein DnaA. We have tested this interpretation more rigorously by using a *dnaA* deletion (null) mutation, *dnaA850::Tn10*, which encodes no detectable DnaA protein. Our results, presented here, show that F cannot be established or maintained in the *dnaA* null host. Thus, we conclude that contrary to prior beliefs, F replication does require the DnaA protein. Furthermore, we speculate that F most likely integratively suppresses *dnaA*(Ts) mutations because they are leaky.

The *dnaA850::Tn10* allele was constructed by insertion of *Tn10* near the amino terminus coding region of the *dnaA* gene and was found to encode no detectable level of DnaA protein (12). It has been shown that the *dnaA::Tn10* allele can replace the wild-type allele if the strain has an *rnh* mutation (22) or if mini-R1 plasmid (pKN500) is integrated into the host chromosome (K. von Meyenburg and T. Kogoma, unpublished observations). Table 1 shows the details of AQ3563 construction. The presence of the *dnaA::Tn10* allele in AQ3563 was confirmed by showing that the Tc^r phenotype could be transduced by P1 *vir* from this strain into an *rnh* strain but could not be transduced into an *rnh*⁺ strain (data not shown). Table 1 contains a list of the strains and plasmids used.

We began our studies by attempting to transform mini-F DNA into the *dnaA850::Tn10* strain AQ3563, but we failed to observe any transformants. This initial failure required us to redo the transformation in such a way that we knew the F DNA was capable of producing transformants and that the AQ3563 strain was competent. To achieve these goals, we designed the experiment shown in Table 2. Plasmid pGA46, a p15 derivative, was added to either mini-F pMF46 or pSC138 DNA, and the mixtures were added to strains AQ3553 [*dnaA5*(Ts)] and AQ3563 (*dnaA::Tn10*) made competent by standard methods (14). Both strains were able to take up and maintain the pGA46 plasmids, but only the

dnaA5(Ts) strain, AQ3553, could maintain mini-F; the null mutant was refractory (Table 2).

Next, we found that native F, i.e., F' plasmids, could not be mated into the *dnaA* null strain. As a positive control to show that the null strain had participated in conjugation, we used a donor cell that would transfer both F and ColE1 plasmids in the same event. Both AQ3553 and AQ3563 accepted the ColE1::TnA plasmid RSF2124, but only AQ3553 retained the F' plasmid. The latter was deduced from the sensitivity of exconjugants (28 of 30, or 93.3%) to the male-specific bacteriophage MS2.

We also found that a mini-F copy number mutant introduced by transduction could not be maintained in AQ3563 but could be maintained in AQ3553 (Table 2). The genetic structure of this element, phi3, is shown in Fig. 1. It is a composite made by insertion of the *plac* Mu3 transposon into pBK117. Both pBK117 and phi3 have copy numbers about sixfold greater than that of the wild type (data not shown).

Conceivably, the host chromosome might exert a *cis* effect on the maintenance of integrated F, thereby obviating the need for DnaA protein. If this were true, then it should be possible to transduce the *dnaA::Tn10* mutation into a *dnaA*(Ts) strain integratively suppressed by an F' element. To test this prediction, P1 *vir* was propagated on either AQ3519 (*dnaA::Tn10*) or DK83 (*tnaA::Tn10*) hosts and then used to transduce a strain integratively suppressed by either mini-R1 (AQ3553) or F'133 (AQ3725). As proof that F'133 was integratively suppressing the conditional *dnaA5* allele in AQ3725, we observed during culture growth at 42°C that this strain could not maintain pSC101, a plasmid that has an absolute dependency on expression of the *dnaA*⁺ allele (5, 8). The *tnaA* locus maps very close to the *dnaA* gene and was used as a positive control for transduction in this region. We found that both AQ3553 and AQ3725 recipients gave a transduction frequency of about 2×10^{-6} (Tc^r colonies per phage) when phage propagated on the *tnaA::Tn10* donor cell were used. Likewise, the *dnaA* null mutation could be transduced into AQ3553 at a frequency of 2.88×10^{-6} , but no Tc^r transductants were detected with the AQ3725 recipient strain (frequency, $<3 \times 10^{-8}$). We interpret these results to indicate that even integrated F cannot be maintained in a host with the *dnaA* null mutation.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype, phenotype, construction, or comment	Reference
Strain		
DK65 ^a	F ⁻ <i>dnaA5 ilv-192 rpoB340 metB1 proA3</i>	12
DK83 ^a	F ⁻ <i>dnaA5 tnaA600::Tn10 rnh-224</i>	12
AQ634 ^a	F ⁻ <i>ilv metB pro</i>	21
AQ2547	As DK65, except <i>rpoB</i> ⁺ <i>metB</i> ⁺	This work ^b
AQ3483	As AQ634, except <i>rnh::cat</i> (AQ634 × P1-SK392, select Cm ^r)	9, this work
AQ3519	As AQ3483, except <i>dnaA850::Tn10</i> (AQ3483 × λ <i>dnaA850::Tn10</i> , select Tc ^r)	12; this work
AQ3553	As AQ2547, except <i>chr::pKN500</i> (AQ2547 × P1-CM1793, select Km ^r at 40°C)	30; this work
AQ3563	As AQ3553, except <i>dnaA5</i> replaced by <i>dnaA850::Tn10</i> (AQ3553 × P1-AQ3519, select Tc ^r)	This work
AQ3723	As AQ2547, except harboring F'133	16; this work
AQ3725	As AQ3723, except <i>chr::F'133</i> (spontaneous integration of F'133)	This work
Plasmid		
pMF46	Ap ^r (see Fig. 1)	11
pSC138	Ap ^r (see Fig. 1)	26
phi3	<i>lac</i> ⁺ Ap ^r ::Mu <i>lac</i> fusion, about 200 base pairs downstream of <i>repE</i> ; transcription of <i>lac</i> ⁺ controlled by <i>repE</i> promoter (see Fig. 1)	This work ^c
pSC101	Tc ^r ; requires <i>dnaA</i> ⁺ for maintenance	
RSF2124	ColE1::TnA (Ap ^r)	25
pGA46	Cm ^r <i>tet</i> ⁺ (Tc ^s); a derivative of plasmid p15	2
pKN500	Mini-R1 (Km ^r)	19
F' <i>lacIP proA</i> ⁺ B ⁺	Lac ⁺ Pro ⁺ (from CSH41, see reference)	17
F'133	F' <i>ilv</i> ⁺ <i>metB</i> ⁺ <i>argH</i> ⁺	16

^a The remaining genotype: *trpA9605 his-29 argH thyA deo metD88*.

^b Various alleles were introduced by generalized transduction with bacteriophage P1 *vir* as described by Lennox (15).

^c The λ *plac* Mu3 virus-transposon system (3) was used to make plasmid phi3 from mini-F pBK117. Phage lambda supplies viral formation genes, but its integration and replication in genes are missing. In vivo, phi3 replication is determined by pBK117.

Our results firmly indicate that F' and various mini-F plasmids cannot be established or maintained in recipient cells containing a *dnaA* null mutation. As viewed by Lane (13) and more recently by Kline (10), native F is a complex plasmid composed of at least two distinct replicons, one of which is itself a complex entity (Fig. 1). Since maintenance of native F is blocked by the null mutation, apparently all subreplicons in F have a dependence on the DnaA protein. A limited but similar conclusion has been made independently by Hansen and Yarmolinsky (Proc. Natl. Acad. Sci. USA, in press), who found that λ Δatt mini-P1 and λ Δatt mini-F ($\Delta oriV$) are not maintained in a strain containing the same *dnaA::Tn10* allele that we used. Their mini-F is similar to pMF46 (Fig. 1). Considering our results and those of Tresguerres et al. (28) alluded to earlier, it appears that

integrative suppression is possible because the conditional *dnaA*(Ts) alleles are leaky. Since the biological activity of the Dna protein is incompletely understood, we cannot tell whether leakiness implies quantitative or qualitative differences in each replicon's usage of the DnaA protein.

Fuller and Kornberg (7) have found a consensus DnaA-binding sequence, TTATCCACA. Furthermore, they have found that variant sequences must match in eight of nine bases to effectively bind DnaA protein (6, 7). Four binding sites are found in the chromosomal origin of replication, *oriC*, and the DnaA protein has been implicated in the control of chromosomal replication (4, 7, 31). Two binding sites (6) are found in the minimal origin of mini-F replication, *oriS*, which has the coordinates 44.944 to 45.161 kilobases (kb) (20). These sites have the sequences TTATCCACT, starting at 44.949 kb, and TTATCCACG, starting at 44.957 kb. We found a third site, TTATCCACT, that starts at 44.564 kb. This site is in a region of F that increases *oriS* efficiency (27). Additionally, we observed that there is a match of seven of nine bases at 45.033 kb in *oriS*. In the complementary strands, there are no nine of nine or eight of nine matches, and there are two seven of nine matches at 44.488 and 44.530 kb. In no case has a role actually been established for any of these sequences in the F plasmid.

Unlike initiation of chromosome replication, in which the DnaA protein may be regulatory, the F *repE* gene product serves this role. Since wild-type cells are able to supply enough DnaA protein to allow at least 28-fold increases in mini-F copy number due to mutation in the *repE* gene (23), the capacity of the cells to synthesize DnaA protein does not appear to limit F replication.

DnaA protein-binding sites are also found in the regions of mini-P1 (1, 6) and pSC101 (7, 29), and the dependency of these plasmids on this protein has been mentioned above. However, it should be pointed out that the existence of such binding sites in other plasmids does not necessarily mean

TABLE 2. Failure of a *dnaA::Tn10* strain to accept F plasmids^a

Experimental stage, donor plasmids ^a	No. of colonies ^b			
	AQ3553 (<i>dnaA5</i>)		AQ3563 (<i>dnaA::Tn10</i>)	
	Cm ^r	Ap ^r	Cm ^r	Ap ^r
Transformation				
pMF46 (Ap ^r) + pGA46 (Cm ^r)	166	360	320	0
pSC138 (Ap ^r) + pGA46 (Cm ^r)	600	100	1,500	0
Transduction [phi3 (Ap^r)]				
		400		0
Conjugation [F' <i>lacIP proA</i>⁺ B⁺ + RSF2124 (Ap^r)]				
		TNTC		TNTC

^a Transformation was done by the method of Lederberg and Cohen (14). Transduction by phi3 was done by using a protocol suitable for lambda phage (24). Conjugation was done by mixing log-phase CSH55FE donor and recipient cells at a ratio of 5 to 1, respectively, in a 1.5-ml sterile Eppendorf centrifuge tube. The mating mixture was pelleted in a microcentrifuge for 60 s, and without further disturbance the pelleted cells were incubated at 37°C for 30 min. The cells were then suspended by vigorous mixing, and samples were plated on L-broth agar plates containing ampicillin and kanamycin or kanamycin and tetracycline to select against the donor strain. Exconjugants were tested for sensitivity to MS2 phage by spotting a drop of virus into a lawn of exponentially dividing cells growing in soft agar. Antibiotics were used at the following concentrations (micrograms per milliliter): ampicillin, 25; chloramphenicol, 25; kanamycin, 40; tetracycline, 10. All media contained kanamycin; additionally, tetracycline was present when AQ3563 was the recipient host for transformation or transduction. All incubations were at 37°C.

^b Values represent the total number of colonies detected in each experiment. TNTC, Too numerous to count.

FIG. 1. Gene map of *EcoRI* f5 fragment of native F. Gene locations and map units are in kilobases. Abbreviations: rep, replication; ori, origin of replication; par, partitioning; ccd, coupled cell division; dnaA, initiator protein for chromosomal replication starting at *oriC*, the chromosomal origin of replication; copB (////), a series of five 19-base-pair repeats that are thought to bind protein E, the product of *repE* (essentially the same repeats are found in *oriS*); x-xx, binding sites for the DnaA protein (A'); C and E, protein products made by the *rep* genes. The *ccd* sequence encodes two proteins that are not shown. References for these observations are found in the review by Kline (10). pSC138 is a mini-F containing the entire *EcoRI* f5 fragment. pMF46 is a mini-F deleted for *oriV* and *repC* (deletion of 40.5 to 43.0 kb) and containing the *Ap*^r transposon Tn3 inserted at 46.130 kb. pBK117 is a mini-F originally structured like pMF46 except that Tn3 was inserted at about 46.019 kb. Subsequent cleavage with *Bam*HI resulted in a deleted replicon containing the indicated F and Tn3 sequences. phi3 is pBK117 with the λ *plac*⁺ Mu3 transposon inserted in Tn3 at 179 base pairs from the F::Tn3 junction at 46.019 and 194 base pairs from the 3' end of the last sense codon in the *repE* gene (Tam and Kline, unpublished data). Note that *bla*⁺ begins at 3.951 kb and terminates at 4.810 kb. Thus, the last 32 base pairs of *bla*⁺ have been insertionally displaced without inactivating the *bla*⁺ gene.

that DnaA is essential for their maintenance. For example, mini-R1 (6) and ColE1 (7) have DnaA-binding sites in their origins but are maintained in the *dnaA* null host (Table 2) (Hansen and Yarmolinsky, in press). Also, to the best of our knowledge, this is the first demonstration that the p15 derivative, pGA46, can be maintained in the *dnaA* deletion host. Knowledge that the DnaA protein does play an essential role in the maintenance of several plasmids will help in ultimately defining the details of plasmid replication and should contribute to understanding of chromosome replication.

This research was supported in part by grants from the National Institutes of Health General Medical Section to B.C.K. (GM25604) and T.K. (GM22092) and in part by a training grant from the National Cancer Institute to J.E.T. and M.S.S. (CA09441).

The technical assistance of Ross Aleff is greatly appreciated.

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