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Similarities in Control of Mini-F Plasmid and Chromosomal Replication in *Escherichia coli*

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In *Escherichia coli*, concentrations of a mini-F plasmid with two origins of replication (*oriV* and *oriS*) were 50% lower in fast-growing cells than in slow-growing cells. By contrast, a mini-F plasmid deleted for *oriV* maintained a uniform concentration in both fast- and slow-growing cells, and in this behavior the plasmid mimicked the control by the host of chromosomal origin (*oriC*) concentration.

Early study of F plasmid concentration indicated that it is very similar to that of the host cell chromosome (9). Subsequently, Collins and Pritchard (5) and Pritchard et al. (25) clearly showed that the ratio of F′ *lac* to the chromosomal origin of replication (*oriC*) decreases as cells grow faster. Since the concentration of *oriC* presumably remains constant (6, 7, 25), it follows that the concentration of plasmid decreases. Pritchard et al. (25) concluded that control of F is different than control of chromosomal replication.

At the time of these studies, F was thought to contain only one set of replication genes. It is now known that F has elements of at least three replicons and that at least two different functional replicons can be derived from F by in vitro recombinant techniques (16, 19, 29). Only the replicon genes located on the *Eco*RI f5 restriction fragment (Fig. 1) produce a plasmid with the maintenance properties of F. In this study, we used two plasmids with the full f5 fragment, pML31 and pSC138, and two derivatives of pML31 (Fig. 1).

The f5 fragment contains two origins of replication. The so-called preferred origin (*oriV*) (8, 17) is dispensable as long as the other origin (*oriS*) remains functional (20). The molecular complexity of F suggests that the results seen by Pritchard and co-workers could well reflect the interplay of the various subreplicons or origins in F.

The smallest f5-derived replicon consists of just *oriS* and *repE* (14, 24). As yet, no one has been able to construct a mini-F plasmid consisting of just *oriV* and the required replication proteins encoded by *repC* and *repE*. Trawick and Kline (32) described a model for *oriS*-directed replication (Fig. 1) in which the *repE* gene product, protein E, acts as both an autorepressor of its synthesis and an initiator of F replication. Subsequently, Tokino et al. (30) and Masson and Ray (21) have shown that protein E binds to F DNA (Fig. 1). In constructing the Trawick-Kline model, the chemical law of mass action was considered sufficient to explain the interactions of protein and DNA. This implies that when cell volume varies as a function of cell growth rate, plasmid concentration should remain constant. Since it does not, at least for F′ *lac* (25, 26) and the f5 mini-F plasmid pML31 (26), the model is either defective or inappropriately applied because these two plasmids contain more replication genes than just *oriS* and *repE*. In this report, we present evidence supporting the latter conclusion.

The plasmids used in this study have been described before (15, 29, 36). Their maps are shown in Fig. 1. The bacterial host for these studies was the *Escherichia coli* K-12 strain CSH50 (23). The method used to measure plasmid concentration has been previously described (26) and is briefly outlined in the legend to Fig. 2.

F′ *lac* and pML31 concentrations drop about 50% as cell-doubling time decreases from 60 to 20 min (25, 26). This analysis was repeated with the mini-F plasmid pSC138 (Fig. 1), and the same result was observed (data not shown). We then tested pMF21, a mini-F plasmid containing just *oriS*, to see if its concentration would be constant, as predicted by the Trawick-Kline model. In this test, eight replicate culture samples with either pML31 or pMF21 were filtered onto the same nitrocellulose filter by the use of manifold filtration (Schleicher & Schuell, Inc.) and processed as a group (Fig. 2). Densitometric analysis of the autoradiogram shown in Fig. 2 is presented in Table 1, experiment 1. A drop in pML31 concentration was seen in fast-growing cells, but no drop was seen for pMF21 in such cells. This is an unprecedented observation; it was confirmed by doing a comparable analysis in a separate experiment with cells containing either pML31 or pMF45, a derivative of pMF21 (Table 1, experiment 2).

The results in Table 1 indicate that pML31 concentration rose at slow growth rates relative to the concentration of pMF21, suggesting that *oriV repC* function is responsible for a rise and not a drop in plasmid concentration. Additionally, the comparisons of experiments 1 and 2 suggest that pMF45 concentration is about twofold greater than that of pMF21. This was confirmed by measuring their relative concentrations when broth cultures bearing either pMF21 or pMF45 were assayed on a single filter (data not shown). The slightly higher concentration of pMF45 must reflect the insertion of Tn*3* into the *copB* locus, mapping between 46.0 and 46.25-kilobases (Fig. 1).

It should be instructive to repeat the above experiments using a mini-F plasmid deleted for *oriS*. Presently it is not possible to construct a "viable" mini-F plasmid that replicates only from *oriV* after inactivation of *oriS*. Tanimoto and Iino (28) have constructed a pBR322:F hybrid that is F $oriV^+$ Δ(*oriS*) that replicates from *oriV* in a *polA* host; however, the presence of pBR322 in this construct and the requirement of a *polA* host precludes its valid use for comparison with our results.

In their original work, Pritchard et al. (25) could only infer that the cell mass/chromosomal origin is constant. In the present work, we examined this premise directly by using plasmid-free CSH50 cultured at a variety of growth rates. The results presented in Table 1, experiment III, show that chromosomal *oriC* concentration in CSH50 was constant. As

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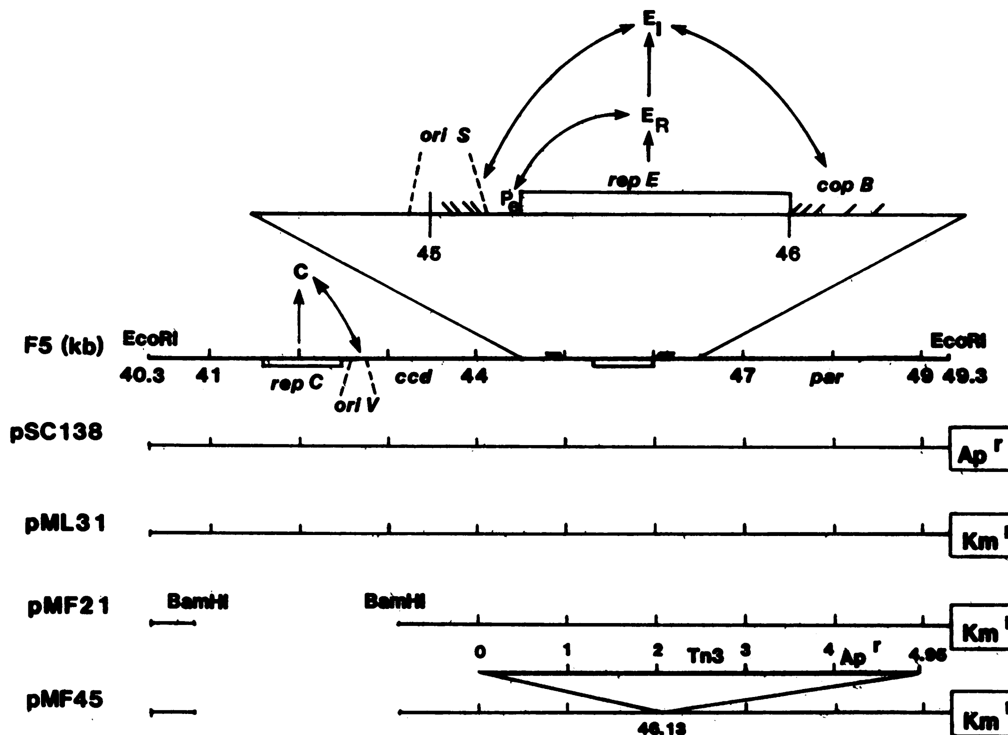

FIG. 1. Various replicons generated from the *Eco*RI f5 fragment of F. Both pMF21 and pMF45 were derived from pML31. The two-stage model for replication control of F is as described by Trawick and Kline (32). Ap, Ampicillin; Km, kanamycin; *cop*, copy number control; *ccd*, coupled cell division; *par*, partitioning; E_R, autorepressor form of the E protein (product of the *repE* gene); E_I, initiator form of the E protein required for replication from *oriS*; C, protein product of the *repC* gene required for replication from *oriV*. Single-ended arrows denote irreversibility of transformation of E_R to E_I, while double-ended arrows denote the equilibrium binding of the two replication proteins to their respective origins and E_I to the iterons (\ and /, direct repeats) found in *orisS* and *copB*. For reviews of mini-F biology, see references 13 and 17.

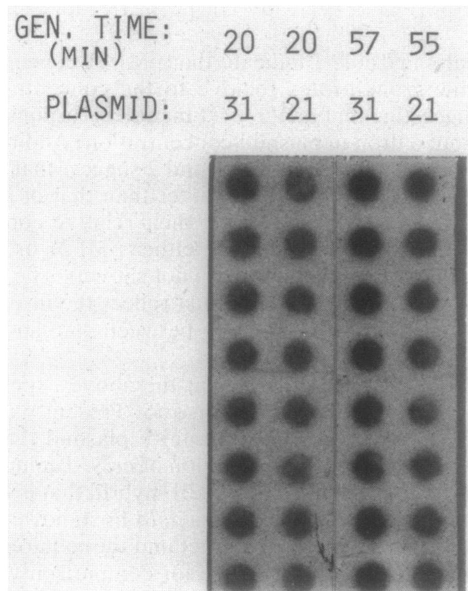

FIG. 2. Relative plasmid concentration as a function of cell growth rate. Bacteria were cultured in L broth (26) for a minimum of 15 generations in the exponential phase to achieve a state of balanced growth. Cells from each culture were then pelleted and suspended in 0.02% NaN_3 M-9 salts (26) to an A_{425} of 0.30, and eight 50-μl samples from each suspension were applied with suction onto a single sheet of nitrocellulose by the use of manifold filtration. Subsequently, all samples were processed for hybridization as previously described (26). ^{32}P-labeled pML31 DNA, prepared by nick translation, was used to probe the released, denatured DNA. Upon completion, the degree of hybridization was quantitated by autoradiography and subsequent densitometric analysis.

discussed by Pritchard et al. (25), the constancy of cell mass/plasmid and cell mass/chromosomal origin ratios suggests that both replicons have similar rules for control of initiation. Our data cannot be used to determine if the initiation masses for the plasmid and chromosome are identical.

Stability of pML31 inheritance. Our assay measures the total plasmid DNA per given amount of cell mass (absorbancy units). If a significant fraction of the cell population were cured of the pML31 plasmid only at the fast growth rates, then this would register as a drop in plasmid concentration. This was not the case in the present study because we found no curing when 200 clones were tested for plasmid loss by replica plating. Additionally, we found that total and viable cell titers agreed within a value of 7% for CSH50 (pML31) cultured at 0.6, 2.3, and 3.0 generations per h (data not shown).

As far as we are aware, the results of this study represent the first documentation that physiological control of the replication of any plasmid can mimic the control of chromosome replication. While the results are consistent with the model we proposed for control involving just F plasmid *oriS*-directed replication, they are not sufficient to prove the

TABLE 1. Plasmid concentration per unit of cell mass measured as a function of growth rate[a]

Expt and avg cell generation time (min)	Concn of plasmid[b]:			Concn of *oriC*[c]
	pML31	pMF21	pMF45	
Expt 1				
20	1.0 ± 0.1	0.9 ± 0.1		
57	1.5 ± 0.2			
55		0.9 ± 0.1		
Expt 2				
22	1.0 ± 0.2		2.3 ± 0.2	
57	2.0 ± 0.2			
61			2.1 ± 0.3	
Expt 3				
22				0.98 ± 0.06
35				0.94 ± 0.08
87				1.00 ± 0.08

[a] Concentrations were determined as outlined in the legend to Fig. 2. Samples within a given experiment were assayed on a single nitrocellulose sheet; thus, all samples within the experiment were processed identically.

[b] The average densitometric value is given for the eight replicated samples from each culture measured by scanning the autoradiogram. The observed densitometric value for a selected plasmid-bearing sample within each experiment was selected as unity (i.e., 1.0). The remaining densitometer values are presented as multiples of the unit value. For all values, the standard deviations are shown.

[c] *OriC gid* amounts per unit of cell mass (plasmid-free CSH50) were determined as outlined for experiments 1 and 2 above and in the legend to Fig. 2, with the exception that a ^{32}P-labeled RNA probe was used. This probe was made in vitro by using pSP64 into which the *oriC gid* region (*Pst*I-*Hin*dIII fragment) had been cloned from pFHC353. pFHC353 was a generous gift from T. Kogoma and has been previously described (36).

model. Our results also suggest that the failure of the mini-F plasmid with two origins (pML31) to maintain a constant concentration at all growth rates is due to *oriV* replication genes. We do not know what property of the *oriV* replication system causes this failure.

Mini-F plasmids that just use *oriS* and *repE* for replication and the *oriC dnaA* replication system have important molecular and genetic similarities. Both have origins containing small repeat sequences (10, 31, 33), and both use an initiator protein that is also an autorepressor of its own synthesis (2, 3, 27, 32). These observations and the demonstration here that both replicons responded similarly to changes in cell physiology suggest that the regulatory mechanisms of the replicons may have more in common than previously believed. It is also worth pointing out that the general molecular motif described above is seen in mini-P1 (1), mini-Rts1 (11), pSC101 (4, 18, 34, 35), R1162/RSF1010 (12), and even the eucaryotic replicon simian virus 40 (for a review, see reference 22). Thus far, none of these replicons have been studied as described here.

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