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Involvement of Integration Host Factor (IHF) in Maintenance of Plasmid pSC101 in *Escherichia coli*: Characterization of pSC101 Mutants That Replicate in the Absence of IHF

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***Escherichia coli* mutants defective in the stable maintenance of plasmid pSC101 have been isolated following Tn*10* insertion mutagenesis. One class of mutations affecting pSC101 replication was located in the genes *himA* and *himD* (*hip*), which encode the two subunits of integration host factor (IHF), a small histonelike DNA-binding protein that has multiple cellular functions. Mutants of pSC101 that could replicate in the absence of IHF were isolated and characterized; four independent mutational alterations were found to affect the third codon of the pSC101 *rep* gene, resulting in the replacement of glutamic acid by lysine. The compensating alteration appears to function by altering the activity of the pSC101 *rep* protein in *him* mutants.**

In nature, bacteria often contain extrachromosomal replicons (i.e., plasmids) that specify functions advantageous to the host, for example, genes that encode antibiotic resistance or confer new metabolic abilities. However, in the absence of selective growth conditions, plasmids must have mechanisms that ensure their inheritance and stable maintenance in populations of dividing cells.

Inheritance of plasmid DNA involves two principal processes. First, the DNA must be replicated prior to cell division, and second, the progeny DNA molecules must be distributed (i.e., partitioned) to daughter cells. Provided that at least one copy of a plasmid replicon is inherited by each daughter cell, a copy number control mechanism ordinarily brings the plasmid copy number to a level characteristic for that particular replicon. Certain plasmids such as ColE1 are thought not to need active partitioning systems because their high copy number makes it unlikely that all plasmid molecules will segregate into the same daughter cell. However, stable maintenance of plasmids having a low or medium copy number requires specific mechanisms to position plasmids with respect to the plane of cell division; sequences that accomplish partitioning have been identified and characterized for such plasmids (1, 21, 37, 45, 47, 57).

We are interested in understanding the mechanisms involved in maintenance in *Escherichia coli* of plasmid pSC101 (8) which exists at four to six copies per chromosome (28) and normally is stably maintained during cell growth. The pSC101 DNA sequence has been determined (5), and the regions involved in replication and partitioning have been characterized. The former includes an origin at which DNA replication initiates and a segment encoding a protein (Rep) required for replication (3, 35, 59). This region also contains a binding site for the host-encoded DnaA protein (18), a product required for pSC101 replication (17, 28) as well as for the initiation of chromosomal replication (29). Numerous host-encoded gene products in addition to DnaA are required for pSC101 replication. These include, but are not limited to, the products of the *dnaB*, *dnaC*, and *dnaG* genes (13, 28).

A segment of pSC101 involved in the partitioning of plasmid DNA molecules between daughter cells is located adjacent to the origin-containing region (37). In the absence of the 375-base-pair (bp), *cis*-acting *par* locus, individual molecules present in the intracellular pool of plasmids do not act as independently assorting units (57). The sequence of the pSC101 *par* locus (38) indicates that it encodes no proteins; however, the *par* region contains three partition-related (PR) segments identified by deletion analysis as being functionally important. The *par* locus has been found to promote membrane binding of pSC101 DNA (27; C. A. Miller and S. N. Cohen, unpublished results) and also to include a strong binding site for DNA gyrase (60). While *par* locus mutations do not directly affect the replication properties of the plasmid, a link between replication and partitioning has been suggested by the finding that some *par* region mutants are unable to compete equally with coexisting *par*$^+$ plasmids despite their normal stability as individual replicons (57).

We have isolated *E. coli* chromosomal mutations that affect the maintenance of the pSC101 plasmid in populations of dividing cells. One class of such Pma$^-$ (plasmid maintenance) mutants resulted from transposon Tn*10* insertions in the *recD* gene (6), which encodes a subunit required for exonuclease V activity of the RecBCD enzyme (2, 6). Mutations in *recD* lead to the formation of concatameric plasmid species, which are not stably maintained (6).

We report here our finding that another class of Tn*10* insertion mutations showing the Pma$^-$ phenotype render the pSC101 plasmid unstable by preventing the synthesis of integration host factor (IHF), a pleiotropic *E. coli* DNA-binding protein shown previously to affect integration and excision of bacteriophage λ, packaging of phage 21 and λ DNA into phage procapsids, fimbrial phase variation (11), DNA transposition (46), replication of phage f1 (24), partitioning of plasmid P1 (19), and both transcriptional and translational control (see Drlica and Rouviere-Yaniv [12] for a review and for other references). Gamas et al. (20) have found independently that IHF mutants are defective in pSC101 replication. We have isolated and characterized plasmid mutations that specifically allow the maintenance of pSC101 in the absence of IHF. Our studies of these plasmid-borne compensatory mutations suggest that IHF is required

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TABLE 1. Multiply-marked strains[a]

Strain	Genotype	Source or reference
AB2829	*aroA354 supE42*	CGSC 2829
GMS343	*aroD6 argE3 lacY1 galK2 man-4 mtl-1 rpsL700 tsx-29? supE44?*	CGSC 5496
Hfr3000	*thi-1 relA1 spoT1 supQ80* Hfr PO1	CGSC 259
JC9239	*thr-1 leuB6* Δ(*gpt proA*)*62 hisG4 recF143 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44 rfbD1 kdgK51 rac*	CGSC 5761
K634	*galK rpsL himA42*	H. Miller (41)
K5242	*galK rpsL himA*Δ*81*	H. Miller
KL188	*pyrD34 trp-45 his-68 thyA25 thi-1 galK2 malA1 xyl-7 mtl-2 rpsL118*	CGSC 4211
PM191	*thi-1 thr-1 fhuA21 deoB deoC supE44 recA56*	37
RW1892	Δ*3*(*himD*)::*cat supE supF*	R. Weisberg
S90C	Δ(*lac pro*) *rpsL*	M. Calos
SC518	*pro met gyr* (R1)	
SC541	*thr-1 leu-6 thi-1 lacY1 fhuA21 supE44* (F::*kan*)	
DPB6	*ara* Val^r Δ(*lac proB*) *galE rpsL thi* [ϕ80 d*lac* with Δ(*lacI tonB trp*)*5*] (pZC1)	6
DPB64	*ara* Val^r Δ(*lac proB*) *galE rpsL thi* [ϕ80 d*lac* with Δ(*lacI tonB trp*)*5*] *himD451*::mini-*tet*	
DPB65	*ara* Val^r Δ(*lac proB*) *galE rpsL thi* [ϕ80 d*lac* with Δ(*lacI tonB trp*)*5*] *himA452*::mini-*tet*	
DPB101	Δ(*lac pro*) *rpsL himD451*::mini-*tet*	
DPB102	Δ(*lac pro*) *rpsL himA452*::mini-*tet*	
DPB254	*ara* Val^r Δ(*lac pro*) *galE rpsL thi* [ϕ80 d*lac* with Δ(*lacI tonB trp*)*5*] *himA453*::mini-*tet* (pZC10)	
DPB316	Δ(*lac pro*) *rpsL himA453*::mini-*tet*	
DPB879	Δ(*lac pro*) *rpsL* (p*himA*$^+$)	
DPB880	Δ(*lac pro*) *rpsL himD451*::mini-*tet* (p*himA*$^+$)	
DPB881	Δ(*lac pro*) *rpsL himA452*::mini-*tet* (p*himA*$^+$)	
DPB882	Δ(*lac pro*) *rpsL himA453*::mini-*tet* (p*himA*$^+$)	
DPB883	Δ(*lac pro*) *rpsL* (pE313 *himD*$^+$)	
DPB884	Δ(*lac pro*) *rpsL himD451*::mini-*tet* (pE313 *himD*$^+$)	
DPB885	Δ(*lac pro*) *rpsL himA452*::mini-*tet* (pE313 *himD*$^+$)	
DPB886	Δ(*lac pro*) *rpsL himA453*::mini-*tet* (pE313 *himD*$^+$)	
Strains derived from MG1655		CGSC 6300 (26)
DPB486	*himD451*::mini-*tet* (pZC14)	
DPB487	*himA452*::mini-*tet* (pZC14)	
DPB565	Δ*3*(*himD*)::*cat himA452*::mini-*tet*	
DPB566	Δ*3*(*himD*)::*cat himA453*::mini-*tet*	
DPB706	*tnaA*::Tn*10 trp*Δ(*EA*)*2*	
DPB719	*recF143 himD451*::mini-*tet*	
DPB720	*recF143 himA452*::mini-*tet*	
DPB736	*recF143* (pZC33)	
DPB737	*recF143 himD451*::mini-*tet* (pZC33)	
DPB738	*recF143 himA452*::mini-*tet* (pZC33)	
DPB854	*lacZ4075*::mini-*tet*Δ(Tc^s) *himD451*::mini-*tet*	
DPB855	*lacZ4075*::mini-*tet*Δ(Tc^s) *himA452*::mini-*tet*	
DPB866	*lacZ4075*::mini-*tet*Δ(Tc^s) (pZC101)	
DPB867	*lacZ4075*::mini-*tet*Δ(Tc^s) *himD451*::mini-*tet* (pZC101)	
DPB868	*lacZ4075*::mini-*tet*Δ(Tc^s) *himA452*::mini-*tet* (pZC101)	
DPB869	*lacZ4075*::mini-*tet*Δ(Tc^s) (pZC102)	
DPB870	*lacZ4075*::mini-*tet*Δ(Tc^s) *himD451*::mini-*tet* (pZC102)	
DPB871	*lacZ4075*::mini-*tet*Δ(Tc^s) *himA452*::mini-*tet* (pZC102)	

[a] All strains are F$^-$ λ$^-$ derivatives of *E. coli* K-12 and were constructed for this study unless otherwise noted. *E. coli* Genetic Stock Center (CGSC) strains were provided by B. Bachmann.

for function of the pSC101 replicase. Characterization of a chromosomal mutation that allows replication of wild-type pSC101 in the absence of IHF is the subject of the accompanying paper (7).

MATERIALS AND METHODS

Strains, media, and general methods. All *E. coli* strains used are derivatives of strain K-12. Full genotypes of multiply-marked strains are listed in Table 1. Strain MG1655 (CGSC 6300), used in many of these studies, is a prototrophic λ$^-$ F$^-$ strain that has not been subjected to multiple rounds of mutagenesis (26). The media used for these studies have been described previously (6). Antibiotics (Sigma) were used at the following concentrations: ampicillin, 10 to 30 μg/ml; tetracycline hydrochloride, 10 μg/ml; kanamycin sulfate, 30 to 75 μg/ml; chloramphenicol, 30 μg/ml; streptomycin sulfate, 100 μg/ml. Isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Boehringer Mannheim) were used at concentrations of 0.5 and 0.1 mM, respectively.

Transductions and transformations were performed as described previously (6). To increase transformation efficiency, $CaCl_2$-treated cultures were usually kept on ice for at least 1 day before transformations were performed. Transformed cells were incubated for 1 to 2 h in liquid LB medium

before being spread on LB plates containing antibiotic to allow for phenotypic expression. Plasmid stability studies were performed as described previously (6). The 50% lethal dose (LD_{50}) determinations for ampicillin on single cells were performed as described by Tucker et al. (57).

Derivatives of strain MG1655 that contained mutation *recF143* were constructed by P1 transduction of strain DPB706 (*tnaA*::Tn*10* *trpΔ*[*EA*]2) with P1 phage grown on strain JC9239 (*recF143*), selecting for the $TnaA^+$ phenotype (i.e., growth on indole [10 μg/ml] in the presence of 50 μg of 5-methyl-tryptophan per ml with 0.2% glycerol as a carbon source). The presence of *recF143* was scored by sensitivity to UV irradiation and also by decreased intramolecular plasmid recombination as measured with plasmid pRDK41 (10).

DNA manipulations and plasmid constructions. Restriction enzymes and DNA-modifying enzymes were from Bethesda Research Laboratories (BRL) or New England Biolabs. Plasmid DNA was isolated by using an alkaline-sodium dodecyl sulfate lysis procedure (36) and was banded twice in CsCl gradients containing ethidium bromide. DNA fragments were isolated from agarose gels by the freeze-squeeze protocol of Tautz and Renz (54). DNA sequencing was performed by using the dideoxy chain-termination method of Sanger et al. (52). Recombinant DNA procedures involving ligations with T4 DNA ligase, making restriction fragment ends blunt with the T4 DNA polymerase or the Klenow fragment of *E. coli* DNA polymerase I, and calf intestinal alkaline phosphatase treatments were performed according to Maniatis et al. (36).

Plasmids encoding Km^r were constructed by inserting a 1.3-kilobase (kb) *Hin*dIII-*Sma*I *aph*-containing fragment of Tn*5* (from plasmid pZT331; D. Biek and J. Roth, unpublished results) into the *Sca*I site in the *bla* gene of various plasmids. The following plasmids were produced in this manner: pZC33 (5.3 kb, Km^r Ap^s pSC101 replicon) derived from pZC14; pZC57 [Km^r Ap^s *rep*(Ts) pSC101 replicon] derived from pEL3 (3); and pSLB20-8 (5.2 kb, Km^r Ap^s pSC101 replicon) derived from pZC20. Plasmids with the designation pSLB were constructed and kindly provided by Serge Beaucage.

Plasmid pSLB61-3 (5.1 kb, Cm^r Ap^s pSC101 replicon) contains the *Hae*II *cat* gene-containing fragment from pACYC184 inserted into the *Sca*I site of pZC20. Plasmid pSLB99 (6.1 kb, Tc^r Ap^s pBR322 replicon) was constructed by elimination of the *Pst*I site in pMC9 (44). The 1.5-kb *Hin*dIII-*Sal*I *aph*-containing fragment of Tn*5* was inserted into *Hin*dIII-*Sal*I-digested pBR322 to give plasmid pZC9 (5.2 kb, Tc^s Km^r Ap^r). Plasmid pZC20 (3.9 kb, Ap^r pSC101 replicon) was constructed by removing the 95-bp *Bam*HI-*Eco*RI *lacUV5* promoter fragment from pPM30 (37), and an *Eco*RI linker was added to regenerate the *Eco*RI site.

Plasmid pZC87 is an 8.6-kb derivative of mini-F containing the 1.3-kb *Eco*RI-*Hae*II *bla* fragment of pPM30 (37) inserted between the *Eco*RI sites of pMF21 (32). The resulting Ap^r Km^s plasmid contains the *lacUV5* promoter region of pPM30 located proximal to the *sopA* gene of pMF21, with an *Eco*RI site at the junction. Plasmids pZC101 and pZC102 (9.7 kb, Ap^r mini-F replicon) contain the 1.07-kb *Hph*I-*Nde*I promoterless *rep* fragment from pZC20 and pZC14, respectively, inserted into the *Eco*RI site of pZC87. The pSC101 *rep* genes in pZC101 and pZC102 are under transcriptional control of the *lacUV5* promoter.

Monitoring the Him phenotype. The presence of mutations in *himA* and *himD* (*hip*) was monitored by one of two tests: failure to allow plaquing of phage Mu*c25* (31, 40), or failure to allow transformation by plasmid pSC101 or its derivatives (this paper).

Isolation of IHF-compensating derivatives of pSC101. Plasmid pPM30 (37), a 4-kb Ap^r plasmid derived from pSC101, was mutagenized by hydroxylamine in vitro, as described by Hashimoto and Sekiguchi (27). Following extensive dialysis to remove hydroxylamine, the DNA was ethanol precipitated and used for transformation of *himA* strain DPB102. Seven independent transformations, each with 100 ng of mutagenized DNA, were performed for each of three mutagenized pPM30 samples. The longest hydroxylamine treatment (20 h at 37°C) resulted in a threefold reduction in transformation efficiency of strain S90C (him^+ parent of DPB102), indicating relatively low levels of mutagenesis.

RESULTS

Isolation of Tn*10* insertions in *pma* genes. Previously we reported the isolation of *E. coli* mutants having mini-*tet* insertions in the chromosome and yielding the Pma^- phenotype (i.e., defective in plasmid maintenance). Chromosomal mutations affecting plasmid pSC101 maintenance were identified by loss of a Km^r $lacI^+$ derivative of pSC101 (pZC1) from a *lacI* $lacZ^+$ strain (DPB6); on X-Gal indicator plates, colonies containing plasmid pZC1 are white and plasmid-free segregants form blue colonies. Four insertion mutations affected the *recD* gene and have been described previously (6). Three other mutations had major effects on transformation by pSC101 and on the ability of pSC101 to be maintained in a population of dividing cells. When P1 phage grown on strains containing these insertions (DPB64, DPB65, and DPB254) was used to transduce the mini-*tet*-generated mutations into strain DPB6, selecting for Tc^r on X-Gal plates, we observed that the transductants formed entirely blue colonies. This suggested that loss of plasmid pZC1 occurred very early during growth of the colony; indeed, when the Tc^r transductants were tested, Km^r clones could not be isolated.

Strain DPB6, in which the mini-*tet* insertions were isolated, was very slow growing, possibly as a result of its deletion of the *tonB* gene. For this reason, the mini-*tet* insertion mutations were moved by P1 transduction into healthier-appearing strains, S90C and MG1655 (26). These transductants were used for all subsequent experiments.

We attempted to introduce pZC1, as well as another pSC101 derivative, pPM30, into the three S90C-derived mutant strains (DPB101, DPB102, and DPB316). While it was not possible to transform the mutants with these pSC101-derived replicons, transformation by other plasmids that we tested (including pBR322, F::*kan*, ColE1::Tn*3*, and R1) occurred at essentially normal frequencies (Table 2). These replicons, unrelated to pSC101, were stably maintained in the mutant strains in the absence of selection (<1% plasmid loss observed after 100 generations of nonselective growth). These results suggested that replication of pSC101 but not of other plasmids is affected by the Pma^- mini-*tet* insertion mutations. All three mutations yielded identical phenotypes with regard to plasmid maintenance.

Tn*10* chromosomal insertions affecting pSC101 replication are located in genes *himA* and *himD*. We suspected that the mutations we had isolated would not be likely to affect any genes required for DNA replication per se, since chromosomal replication, as well as replication of other plasmids we have tested, was not noticeably altered. The results of Hfr crosses in which the Pma^- mutants were used as recipients (data not shown) suggested that two of the three mini-*tet* insertions, those in the Pma^- strains DPB102 and DPB254,

TABLE 2. Effect of chromosomal Pma⁻ mutations on maintenance of various plasmids

Recipient strain	Phenotype	Transformability and stability of indicated plasmids[a]				
		pZC1	pPM30	pBR322	R1	F::*kan*
S90C	Pma⁺	+	+	+	+	+
DPB101	Pma⁻	−	−	+	+	+
DPB102	Pma⁻	−	−	+	+	+
DPB316	Pma⁻	−	−	+	+	+

[a] Plasmids pPM30, pBR322, and pZC1 were introduced by transformation, selecting for Ap^r for pPM30 and pBR322 and Km^r for pZC1. Plasmids R1 and F::*kan* were introduced by conjugation (from strains SC518 and SC541, respectively), selecting for Km^r on minimal glucose plates containing proline. For the Pma⁻ recipients, + indicates the ability to introduce plasmids at frequencies approximately equal to that obtained with the isogenic *him*⁺ strain S90C, while − indicates that no transformants were obtained (>10^5-fold reduction relative to S90C). Plasmids that could be introduced into strains (indicated as +) were maintained stably in the absence of selection (>99% of the cells retained the plasmid after 100 generations of growth).

were located in the 37-min region of the chromosome (refer to Bachmann [4] for a genetic map of *E. coli* K-12). Transduction studies with P1 phage grown on the mutants showed that the insertion mutations in strains DPB102 and DPB254 were linked to *aroD* (40 to 55% P1 cotransduction with *aroD6* in strain GMS343), while the mini-*tet* insertion in Pma⁻ strain DPB101 was not detectably linked to *aroD* (<0.7% cotransduction). During tests for linkage of the mini-*tet*-generated mutations to the *himA* gene, which is located in this region (41), we learned of an earlier observation that pSC101 could not be introduced into *himA* mutants (H. Miller, personal communication). It therefore appeared possible that the mutations linked to *aroD* might be located in the *himA* gene, which encodes the α subunit of the heterodimeric protein IHF (43).

The β subunit of IHF is encoded by the *himD* gene (also refered to as *hip*) (16). To test whether the mini-*tet* insertion in strain DPB101 that we had identified as being unlinked to *himA* (*aroD*) was located in the *himD* gene, we tested for transductional linkage between the mutation and the *pyrD* and *aroA* genes, which are located near *himD* (at 20 to 21 min on the chromosome [4]). We found that the mini-*tet* insertion in DPB101 was 11% P1 cotransducible with *pyrD34* (in strain KL188) and greater than 95% cotransducible with *aroA354* (in strain AB2829). Our Pma⁻ mini-*tet* insertions were cotransduced with *aroD*, *aroA*, and *pyrD* at frequencies similar to those of known *him* mutations.

The results of several studies summarized below confirmed that the mutations we isolated on the basis of their effects on pSC101 maintenance are in fact located in the previously identified genes *himA* and *himD*. First, known mutations in these *him* genes (*himA42* [strain K634], *himAΔ81* [strain K5242], and *Δ3(himD)::cat* [strain RW1892]) affected replication of pSC101 as did the mini-*tet* mutants we isolated with the Pma⁻ phenotype (data not shown). Second, both the *him* mutants, in which phage Mu replication is blocked because of a failure to express Mu early genes (22, 33), and these Pma⁻ mutants did not allow plating of Muc*25* phage (Table 3). Third, and most importantly, complementation tests with pBR322 derivatives containing the cloned *him* genes (p*himA*⁺, provided by H. Miller, and pE313, carrying *himD*⁺ [16], provided by R. Weisberg) indicated that the failure to allow pSC101 replication as well as the failure to allow plaquing by phage Muc*25* were complemented by the corresponding cloned gene (Table 3). The complementation results suggested that the mutations are recessive, as expected for insertion mutations, which commonly abolish activity of the gene into which they have inserted. The mutations have been named *himD451*, *himA452*, and *himA453*.

Isolation and characterization of compensating mutations that allow pSC101 replication in the absence of IHF. To elucidate the role that IHF plays in pSC101 replication, we isolated plasmid mutations that allowed pSC101 replication in *him* mutants. We lightly mutagenized plasmid pPM30, an Ap^r derivative of pSC101 (37), with hydroxylamine (see Materials and Methods) and introduced the plasmid by transformation into the *himA* mutant DPB102. Seven Ap^r pPM30 transformants of DPB102 were obtained. Transformation of the isogenic *him*⁺ strain S90C by the same mutagenized DNA preparations yielded approximately 10^5 Ap^r transformants. Controls with unmutagenized pPM30

TABLE 3. Complementation tests with the plasmid maintenance mutations and cloned *him* genes

Strain[a]	Relevant markers[b]	Resident plasmid	pSLB20-8 transformation[c]	Plating of phage Muc*25*[d]
S90C	*him*⁺ (Pma⁺)	None	+	+
DPB101	*himD451*::mini-*tet* (Pma⁻)	None	−	−
DPB102	*himA452*::mini-*tet* (Pma⁻)	None	−	−
DPB316	*himA453*::mini-*tet* (Pma⁻)	None	−	−
DPB879	*him*⁺	p*himA*⁺	+	+
DPB880	*himD451*::mini-*tet*	p*himA*⁺	−	−
DPB881	*himA452*::mini-*tet*	p*himA*⁺	+	+
DPB882	*himA453*::mini-*tet*	p*himA*⁺	+	+
DPB883	*him*⁺	pE313 (*himD*⁺)	+	+
DPB884	*himD451*::mini-*tet*	pE313 (*himD*⁺)	+	+
DPB885	*himA452*::mini-*tet*	pE313 (*himD*⁺)	−	−
DPB886	*himA453*::mini-*tet*	pE313 (*himD*⁺)	−	−

[a] All strains are derivatives of S90C.

[b] Based on these and other studies (described in text), these mutations resulting in the Pma⁻ phenotype have been found to affect the genes *himA* and *himD*.

[c] Transformations were performed with 2 ng of pSLB20-8 plasmid DNA and selection on kanamycin (75 μg/ml). Plasmid pSLB20-8 is a Km^r derivative of pSC101. Symbols: +, transformation with efficiencies at least 80% of that observed with strain S90C; −, no transformants obtained (>10^3-fold reduction relative to S90C). All strains were transformable with similar frequencies by the unrelated replicon pZC9 (a Km^r derivative of pBR322).

[d] Phage Mu plating was performed by incubating cultures with dilutions of Muc*25 vir* lysates and plating in soft agar on LB plates. Symbols: +, Mu plating efficiencies similar to those observed for strain S90C; −, no plaques visible (>10^3-fold reduction relative to S90C).

TABLE 4. Transformation of *him* mutants by IHF-compensating mutants of plasmid pSC101

Strain[a]	Relevant markers	Relative frequency of Ap^r transformants[b]				
		pPM30	pZC14	pZC15	pZC16	pZC17
MG1655	*him*$^+$	1.0	1.0	1.0	1.0	1.0
DPB269	*himA452*::mini-*tet*	<0.001	0.27	0.31	0.27	0.26
DPB272	*himA453*::mini-*tet*	<0.001	0.36	0.27	0.28	0.33
DPB268	*himD451*::mini-*tet*	<0.001	0.31	0.21	0.31	0.47
DPB564	Δ3(*himD*)::*cat*	<0.001	0.23	0.27	0.28	0.39
DPB565	*himA452*::mini-*tet* Δ3(*himD*)::*cat*	<0.001	0.35	0.22	0.22	0.17
DPB566	*himA453*::mini-*tet* Δ3(*himD*)::*cat*	<0.001	0.36	0.50	0.42	0.41

[a] All strains are derivatives of MG1655.

[b] Transformations were performed with 2 to 5 ng plasmid DNA and selection on ampicillin (20 μg/ml). The results represent the means from two experiments and are expressed as the frequency of transformants obtained relative to the frequency with the *him*$^+$ strain MG1655. The frequencies have been adjusted for differences in ability of the individual strains to serve as transformation recipients as measured by transformation with the pBR322-derived plasmid pZC9 in the same experiment.

plasmid DNA yielded no Ap^r transformants with DPB102. Of the seven Ap^r transformants of DPB102 obtained after transformation with mutagenized pPM30 plasmid DNA, six were from independent transformations; only these six were chosen for further study.

Plasmid DNA isolated from the DPB102 Ap^r transformants, which was indistinguishable from pPM30 by restriction analysis (data not shown), was tested for ability to retransform DPB102. Of the six pPM30 derivatives, four (pZC14 to pZC17) were able to transform DPB102 and thus must contain mutations on the plasmid that allow transformation of *himA* strains. Unexpectedly, plasmid DNA from two strains was not able to transform DPB102; the strains from which these plasmids were isolated contained a spontaneous chromosomal mutation that compensated for the absence of IHF and allowed transformation by wild-type pSC101-derived plasmids. Studies of this chromosomal mutation are described in the accompanying paper (7).

The plasmid mutations compensating for the defect in IHF were isolated in the *himA452*::mini-*tet* insertion strain DPB102; however, they also replicated in *himD* mutants as well as *himA himD* double mutants (Table 4). This finding suggests that residual IHF function encoded by one of the *him* genes is not required and implies that replication of the mutant plasmids can occur in the complete absence of IHF.

While the IHF-compensating mutant plasmids (pZC14 to pZC17) were able to transform *him* strains efficiently, we noticed that they were not maintained with normal stability in the *him* mutants. In wild-type (*him*$^+$) strains, both the mutant plasmids and the wild-type parent plasmid were stably maintained (Table 5). In contrast, in *him* mutants, 85% of the cells lost the IHF-compensating plasmid after 20 generations of nonselective growth, and approximately 96% of the cells lost the plasmid after 40 generations (Table 5). In addition, the level of ampicillin resistance conferred by the *bla* gene, which is directly proportional to the copy number of the gene (58), was lower in *him* mutants than in the wild type. (In *him*$^+$ strains, the mutant plasmids conferred LD_{50} ampicillin resistance levels that were very slightly higher than that of the parent plasmid, pPM30 [Table 5]). All four compensating mutants showed identical properties in these tests. Thus, while the plasmid mutations we isolated allowed replication in the absence of IHF, they did not completely circumvent the need for IHF.

Plasmid-borne compensating mutations function in *trans*. To determine whether the mutations present in plasmids pZC14 to pZC17 allow pSC101 replication in the absence of IHF by providing a *trans*-acting function or act by altering *cis*-acting sequences, a *cis-trans* test was performed in which *him* mutant strains containing an IHF-compensating mutant plasmid were transformed with wild-type (i.e., non-IHF-compensating) derivatives of pSC101. To preclude recombination between the resident pSC101 plasmid and the introduced plasmid, we introduced a *recF* mutation, which renders the host specifically deficient in plasmid recombination, into the recipient strains (30).

As can be seen in Table 6, the presence of the IHF-compensating mutant plasmid pZC33 (a Km^r derivative of pZC14 containing the *aph* gene from Tn*5* inserted into the *bla* gene of pZC14) in *himA* or *himD* strains allowed transformation by wild-type (noncompensating for IHF deficiency) pSC101-derived plasmid pPM30. Similar results were obtained for the other three IHF-compensating mutant plasmids, as well as for plasmids pZC14 to pZC17 when a Km^r pSC101 derivative, plasmid pSLB20-8, was introduced in *trans* in *him* strains (data not shown). Transformants from the experiment shown in Table 6, when plated nonselectively, segregated Ap^s derivatives that were also Km^r and Ap^s derivatives that were also Km^s; they did not segregate Ap^r Km^s derivatives, consistent with the notion that maintenance of the introduced wild-type Ap^r plasmid required a product provided in *trans* from the resident Km^r IHF-compensating mutant plasmid.

The Ap^r Km^r *him* transformants, which contained both mutant and wild-type pSC101 derivatives, grew slowly on

TABLE 5. Copy number and stability characteristics of IHF-compensating mutant plasmids[a]

Strain	Relevant marker and plasmid	Ampicillin LD_{50}[b] (μg/ml)	% of cells with plasmid[c] (no. of generations)
DPB484	*him*$^+$ (pPM30)	114	100 (100)
DPB485	*him*$^+$ (pZC14)	128	100 (100)
DPB486	*himD451* (pZC14)	33	14 (20)
DPB487	*himA452* (pZC14)	32	15 (20)

[a] The strains used are derivatives of MG1655. All four IHF-compensating mutant plasmids (pZC14 to pZC17) behaved similarly in these tests, and results are only shown for pZC14.

[b] Single-cell Ap^r levels were determined as described before (6). The level of Ap^r conferred is related to the copy number of the *bla* gene (58) present on pPM30 and its derivatives pZC14 to pZC17. Results are the mean values from two determinations.

[c] Plasmid stability studies were performed as described before (6), and values represent the fraction of cells containing plasmid after the indicated number of generations of growth in the absence of selection for the plasmid. Results are means from two determinations. These values have not been corrected for the fraction of cells in the initial colonies on ampicillin plates (i.e., at generation zero) that did not contain plasmid, which for strains DPB486 and DPB487 was 15 and 13%, respectively.

TABLE 6. *cis-trans* test for IHF-compensating plasmid mutations[a]

Strain	Relevant genotype	Selected marker	Relative transformation frequency[b]			
			pPM30	pZC14	pEL3	
					30°C	42°C
DPB718	*him*$^+$	Ap^r	1.0	1.0	1.0	<0.001
DPB719	*himD451*	Ap^r	<0.001	0.95	<0.001	<0.001
DPB720	*himA452*	Ap^r	<0.001	0.92	<0.001	<0.001
DPB736	*him*$^+$ (pZC33)	Ap^r	0.55	0.75	0.81	0.71
DPB736	*him*$^+$ (pZC33)	Ap^r Km^r	0.67	0.74	0.83	0.67
DPB737	*himD451* (pZC33)	Ap^r	0.25s	0.59	0.21s	0.27s
DPB737	*himD451* (pZC33)	Ap^r Km^r	0.27s	0.34s	0.24s	0.33s
DPB738	*himA452* (pZC33)	Ap^r	0.36s	0.87	0.39s	0.36s
DPB738	*himA452* (pZC33)	Ap^r Km^r	0.37s	0.35s	0.38s	0.35s

[a] All strains are derivatives of MG1655 and contain the mutation *recF143* to prevent recombination between introduced and resident (pZC33) plasmids. Plasmid pZC33 is a Km^r derivative of IHF-compensating mutant plasmid pZC14. Plasmid pPM30 is an Ap^r derivative of wild-type pSC101 (37), and pEL3 is an Ap^r *rep*(Ts) derivative of pSC101 (3).

[b] Recipient cultures were grown in LB or LB with kanamycin (30 μg/ml) (for pZC33-containing strains); 3 ng of plasmid DNA was used per transformation. Prior to phenotypic expression, the pEL3 transformation mixtures were split in half and incubated at 30 or 42°C and then plated at these temperatures. Transformants were selected on ampicillin (8 μg/ml) or ampicillin plus kanamycin (8 and 30 μg/ml, respectively) to select for maintenance of pZC33. Results are expressed as the frequency of transformants relative to the frequency obtained with strain DPB718 (*him*$^+$) (at 30°C for pEL3) and have been corrected for differences in the ability of the individual strains to serve as transformation recipients as measured by transformation with the pBR322 derivative pZC9 in the same experiment. The results are from a single experiment; however, repeat experiments gave similar values. An "s" after the number indicates that the transformant colonies were small and required an extra day of incubation to grow to the usual size.

selective medium, requiring an extra day of incubation, consistent with the observation that the compensating mutant plasmids are maintained at lower than normal copy number in *him* strains (Table 5). The presence of two differentially marked IHF-compensating mutant plasmids in the same *him* strains also resulted in slow growth when both plasmids were selected (Table 6).

Plasmid DNA isolated from the transformants was examined by agarose gel electrophoresis (Fig. 1). Two pSC101-derived species of molecules were present in each transformant, and there was no evidence of recombination products, except in one instance (1 of 10 *him* transformants tested, lane 15), in which a recombinant plasmid was observed in addition to both of the individual plasmids. We hypothesize that this transformant initially may have contained pPM30 maintained in *trans* with pZC33, but that a recombination event (which may have been selected because of the slow growth of *him* strains that contain both plasmids as separate replicons) occurred during subsequent growth. The amount of the *trans*-complemented wild-type pPM30 replicon in these strains was reduced relative to that of the IHF-compensating plasmid pZC33. The cultures used for isolation of these plasmid preparations were grown during selection for both plasmids (Ap^r and Km^r). When the *him* transformants were grown during selection for only the wild-type Ap^r pPM30 plasmid, the amount of pPM30 was very similar to the amount of pZC33 that was present (not shown). The observations may reflect the relative copy number within the cellular pool of each plasmid required to confer resistance to the particular concentrations of ampicillin and kanamycin used.

The Ap^r transformants contained pPM30 plasmids that were shown to still require IHF; i.e., they had not acquired an IHF-compensating mutation by recombination or mutation. Except for the one *himA* transformant mentioned above that contained some pPM30-pZC33 recombinant molecules, plasmid DNA from the transformants retransformed *himD451*::mini-*tet* strain DPB268 very poorly relative to the isogenic *him*$^+$ strain MG1655 (data not shown). The few Ap^r transformants of DPB268 that were obtained were all weakly Km^r (i.e., had also inherited the IHF-compensating plasmid pZC33). These results indicate that wild-type pSC101 was maintained in *him* strains if an IHF-compensating mutant plasmid was present to provide some *trans*-acting function.

Complementation in *trans* was also observed in *himA*

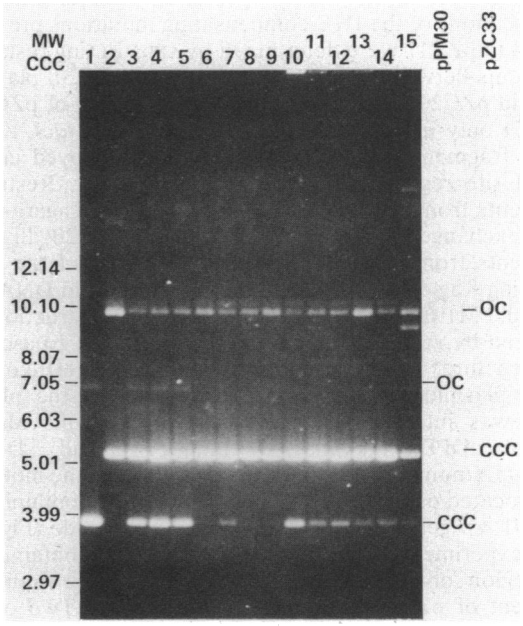

FIG. 1. Plasmid DNA isolated from transformants from the *cis-trans* test (Table 6), demonstrating the presence of both pPM30 and pZC33. Individual transformants were picked from medium selective for both plasmids and streaked for single colonies. Cultures containing ampicillin and kanamycin were grown, and plasmid DNA was isolated and run on a 0.7% agarose gel in 100 mM Tris-borate (pH 8.3)–2 mM EDTA. Molecular size markers from a supercoiled ladder (BRL) are indicated (in kilobases) at left. Lane 1, pPM30; lane 2, pZC33; lanes 3–15, pPM30 transformants of the following strains: lanes 3–5, DPB736 [*him*$^+$ (pZC33)]; lanes 6–10, DPB737 [*himD451* (pZC33)]; lanes 11–15, DPB738 [*himA451* (pZC33)]. The species migrating in front of nicked circular pZC33 in lane 15 is a recombinant between pZC33 and pPM30. The positions of supercoiled (CCC) and nicked circular (OC) forms of pPM30 and pZC33 are indicated at the right.

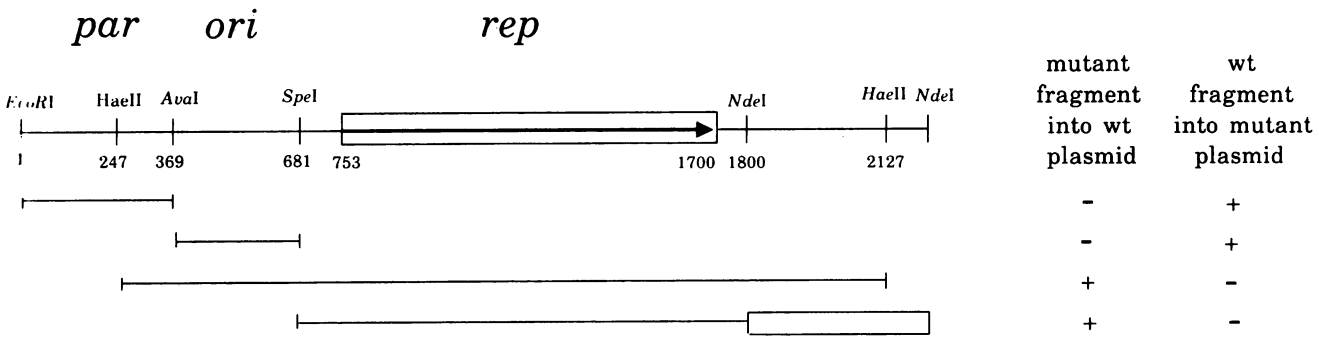

FIG. 2. Mapping of the IHF-compensating mutations present on pZC14 to pZC17 by substituting restriction fragments between mutant and wild-type (wt) (pZC20) plasmids. The lines indicate the extent of the plasmid sequences exchanged between plasmids. Numbers below the line indicate the distance (in base pairs) from the *Eco*RI site (originally a *Hin*cII site in pSC101 adjacent to the *par* locus [37]). Constructions were performed in *him*$^+$ strain pM191, and the hybrid plasmids were tested for transformation of *him* mutants DPB268 (*himD451*::mini-*tet*) and DPB269 (*himA452*::mini-*tet*); the results are indicated at the right. The box in the *Spe*I-*Nde*I plasmids represents a deletion of a 350-bp *Nde*I fragment, which has no observed effect on plasmid replication for either wild-type or IHF-compensating mutant plasmids.

recF$^+$ and *himD recF*$^+$ strains containing a resident IHF-compensating mutant plasmid (data not shown); surprisingly however, no complementation was observed in *himA recA* and *himD recA* mutants. The compensating mutant plasmids were maintained individually in such *him recA* strains, but two compensating mutant plasmids also were not maintained together. We do not know the basis for these *recA*-dependent effects.

Localization of the IHF-compensating plasmid mutations. The locations of the IHF-compensating mutations present in pZC14 to pZC17 were determined by substituting restriction fragments between mutant and wild-type (pZC20) plasmids. Plasmid pZC20 differs from pPM30 (the parent of pZC14 to pZC17) only in that the 95-bp *Bam*HI-*Eco*RI *lacUV5* promoter fragment of pPM30 (37) has been removed and the *Eco*RI site regenerated with an *Eco*RI linker. Restriction fragments from pZC14 to pZC17 purified from agarose gels were exchanged for their counterpart in pZC20; likewise, fragments from pZC20 were ligated in place of the corresponding fragments in pZC14 to pZC17. Plasmid DNA from several PM191 transformants from each construction was analyzed by restriction endonuclease digestion to ascertain the structure. To score for presence of the IHF-compensating plasmid mutations in these plasmids, the plasmid DNA was introduced into the isogenic strains MG1655 (*him*$^+$), DPB268 (*himD451*::mini-*tet*), and DPB269 (*himA452*::mini-*tet*). Our results indicated that the mutations were located on a 1.1-kb *Spe*I-*Nde*I fragment containing the pSC101 *rep* gene and about 100 bp on either side (Fig. 2).

An experiment in which the *Hae*II fragment containing the *rep* region of pZC14 was substituted for the equivalent fragment of pZC20 gave unexpected results. Two of four plasmids tested were able to transform *him* mutant strains and two were not. The plasmids that transformed *him* strains contained the *Hae*II fragments in the normal orientation, while those which could not transform *him* mutants contained these *Hae*II fragments in reverse orientation. A previously characterized plasmid (pWTT310) of similar structure containing this *Hae*II region in reverse orientation appeared to be the same as the wild type with respect to plasmid stability and Cmp phenotype (57). For reasons that presently are not understood, our plasmids containing the *Hae*II fragment in reverse orientation were unable to compete equally with wild-type pSC101-derived plasmids in *him*$^+$ strains (i.e., were Cmp$^-$ [57]) (C. Miller, unpublished results); we suspect that their ability to replicate may be compromised in some way that is made more severe by mutations in IHF.

The 1.1-kb *Spe*I-*Nde*I *rep* fragments from plasmids pZC14 to pZC17 and pPM30 were cloned in M13mp18 (48), and the DNA sequence was determined beginning at the *Spe*I end. At a position 78 nucleotides from the *Spe*I site, we found an identical mutational alteration present in all four IHF-compensating mutants (Fig. 3). This G-to-A transition in the third codon of the *rep* gene is expected to result in the replacement of glutamic acid by lysine. The wild-type sequence we determined for this region agrees with that reported by Vocke and Bastia (59). The mutational alteration is consistent with the known action of hydroxylamine, which produces GC-to-AT transitions. This change was the only difference in any of the mutants in the region between the *Spe*I site and about 100 bp distant in the *rep* gene. We have not determined the sequence of the remaining 1-kb region between this and the *Nde*I site, which delimits the boundary of our mapping results; however, the finding that all four independently isolated compensating mutant plasmids (pZC14 to pZC17) contain an identical alteration is strong evidence for the view that this mutation is causally responsible for the IHF-compensating properties of the plasmid.

Is alteration in the Rep protein sequence mechanistically implicated in IHF compensation? The finding that the IHF-compensating mutation is *trans* acting and is located in the *rep* coding sequence implies that the mutation acts by affecting the Rep protein. However, the change accomplished by the compensatory mutation potentially could affect the Rep protein structure or, alternatively, affect production of Rep protein. If alteration of Rep protein primary structure is important, the role of IHF in pSC101

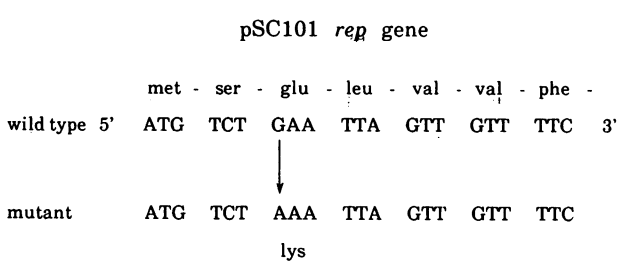

FIG. 3. Nucleotide sequence of the beginning of the *rep* gene of pSC101 and IHF-compensating mutants pZC14 to pZC17. All four mutant plasmids contain the same alteration in the *rep* gene.

TABLE 7. Importance of *rep* alteration in IHF compensation

Strain	Relevant genotype[a]	Relative frequency of Km^r transformants[b]			
		pSLB20-8		pZC57 + IPTG	
		With IPTG	Without IPTG	30°C	42°C
DPB853	*him*$^+$	1.0	0.98	1.0	<0.002
DPB854	*himD451*	<0.003	<0.003	n.d.[c]	n.d.
DPB855	*himA452*	<0.004	<0.004	n.d.	n.d.
DPB866	*him*$^+$ [pZC101 (*rep*^{wt})]	1.08	1.35	3.9	3.1
DPB867	*himD451* [pZC101 (*rep*^{wt})]	<0.003	<0.003	n.d.	n.d.
DPB868	*himA452* [pZC101 (*rep*^{wt})]	<0.002	<0.004	n.d.	n.d.
DPB869	*him*$^+$ [pZC102 (*rep*^{IHF})]	1.03	0.99	4.3	4.4
DPB870	*himD451* [pZC102 (*rep*^{IHF})]	0.46	0.26t	n.d.	n.d.
DPB871	*himA452* [pZC102 (*rep*^{IHF})]	0.55	0.22t	n.d.	n.d.

[a] All strains are derivatives of MG1655. Plasmid pZC101, a derivative of mini-F, contains the wild-type pSC101 *rep* gene fused to the *lacUV5* control region. Plasmid pZC102 contains the IHF-compensating mutant *rep* gene fused to the *lacUV5* control region.

[b] Plasmid pSLB20-8 is a Km^r derivative of pZC20 (wild-type pSC101), and pZC57 is a Km^r derivative of pEL3 [*rep*(Ts) pSC101 (3)]. Transformations were performed with 2 ng of plasmid DNA. At the time of phenotypic expression, the pSLB20-8-transformed cultures were split in half, and IPTG was added to 0.5 mM for one of each pair. For plasmid pZC57, transformed cultures were split in half and placed at 30 or 42°C. Platings were on kanamycin (50 μg/ml) with and without 0.5 mM IPTG (at 30 or 42°C for pZC57 transformations). The results represent the means from two experiments and are expressed as the frequency of transformation with a recipient relative to the frequency obtained with *him*$^+$ strain DPB853 plus IPTG (at 30°C for pZC57). The frequencies have been adjusted for differences in ability of the individual strains to serve as transformation recipients as measured with the pBR322-derived plasmid pZC9. A "t" after the number indicates that the transformant colonies were tiny and required an extra day of incubation to even become visible. The *lac* promoter in pZC101 and pZC102 is not completely repressed in these strains even in the absence of IPTG.

[c] n.d., Not determined.

replication may be to interact with Rep protein either directly or indirectly. This might occur in a manner analogous to the action of IHF in λ integration, in which IHF and Int protein interact with *attP* sequences to form a complex nucleoprotein structure (50, 56).

To investigate these possibilities, we introduced pSC101 into *him* mutant strains in which wild-type Rep protein was produced under the control of a heterologous promoter on a compatible mini-F replicon. Replication of the wild-type pSC101 in such strains would indicate that the amino acid sequence change present in the Rep protein in the IHF-compensating mutants is not the basis for the effects of the compensatory mutations. To perform this experiment, we cloned the *Hph*I-*Nde*I fragment containing the wild-type or IHF-compensating mutant *rep* gene (*rep*^{wt} and *rep*^{IHF}, respectively) lacking its normal promoter in mini-F plasmid pZC87 under control of the inducible *lacUV5* promoter. Constructs in which a heterologous promoter is fused at the site of *Hph*I cleavage in the *rep* gene promoter region are not regulated by Rep protein (61).

The results of this experiment (Table 7) showed that only the mutant *rep*^{IHF} gene (on plasmid pZC102) allowed transformation of a Cm^r pSC101 derivative, pSLB61-3, in *him* mutant strains. Both the *rep*^{IHF} and *rep*^{wt} mini-F plasmids (pZC102 and pZC101, respectively) could provide Rep protein in *trans* in the presence of IPTG to allow replication of a *rep*(Ts) plasmid (pEL3 [3]) in *him*$^+$ strains, indicating that each is able to produce functional Rep protein (Table 7). [Under noninducing conditions, sufficient Rep protein is synthesized in these *him*$^+$ strains to allow replication of the *rep*(Ts) plasmid pEL3 at 42°C. However, in the presence of excess *lac* repressor (produced by plasmid pSLB99, a derivative of pBR322 carrying the *E. coli lacI*$^+$ gene) replication of pEL3 at 42°C only occurs in the presence of IPTG, indicating that the *rep* genes present in both pZC101 and pZC102 are under *lac* control.] These findings demonstrate at least two important points. (i) The product that enables replication of compensating mutant pSC101 replicons in *him* mutant strains is an altered Rep protein and not, for example, an RNA molecule transcribed in the opposite direction from the *rep* gene. (ii) The mutation that compensates for the absence of IHF does not function by creating a new promoter that allows transcription of *rep*, since the *rep*^{IHF} gene in pZC102 is repressed in the presence of high levels of *lac* repressor. These results support the view that alteration of the Rep protein itself enables pSC101 replication in the absence of IHF.

DISCUSSION

IHF was initially identified as a histonelike DNA-binding protein of *E. coli* (16, 39) required for site-specific recombination between phage λ and bacterial attachment sites (see Drlica and Rouviere-Yaniv [12] for a review). IHF is known to participate in a number of other biological processes in addition to λ integration, as noted above. In the studies reported here, we have found that a class of *E. coli* mutations that affect maintenance of the pSC101 plasmid map in the genes encoding IHF subunits.

Mutants of pSC101 that were able to replicate in the absence of IHF also allowed replication of coexisting wild-type pSC101 plasmids in *trans* in *E. coli him* mutants, indicating that the mutational alteration affects a diffusible product required for pSC101 replication. The mutation associated with IHF compensation changed the third codon of the pSC101 *rep* gene and resulted in the substitution of lysine for glutamic acid in the Rep protein. The protein alteration is located in a region of potential secondary structure identified by Vocke and Bastia as a helix-turn-helix motif (59).

Our results indicate that it is an alteration of the primary structure of the Rep protein that accomplishes the observed compensation for the absence of IHF. Production of the mutant Rep protein, but not the wild type, under control of an inducible *lac* promoter allowed replication of wild-type pSC101 in *trans* in *him* strains. There is no reason to believe that IHF affects *rep* gene expression, since a *rep*::*lacZ* gene fusion shows equivalent levels of expression in *him*$^+$ and *him* mutant strains (20; our unpublished results). While the

mutant and wild-type plasmids are maintained at similar copy numbers and show normal incompatibility properties in *him*$^+$ strains, a phenotypic difference was observed between the mutant and wild-type *rep* genes in a *him*$^+$ host when the genes were put under control of the *lac* promoter-operator region. In strain PM191, under *lac*-repressed conditions, the mutant *rep* gene in pZC102 allowed replication of a coexisting *rep*(Ts) pSC101 derivative (pEL3 [3]) in *trans* at the nonpermissive temperature, whereas the wild-type *rep* gene on pZC101 did not allow replication of the coexisting *rep*(Ts) derivative unless expression from the *lac* promoter was induced by treatment with IPTG. This may indicate that low levels of the mutant Rep protein are more active than the wild-type Rep protein.

We have briefly reported our finding of the requirement of IHF for replication of pSC101 previously (9). Gamas et al. (20) have reported their independent finding of the requirement for IHF in pSC101 replication, a discovery made during studies of IS*1* transposition. They demonstrated that IHF is not required for expression of the *rep* gene. Our findings concerning the mode of action of the IHF-compensating mutation are consistent with this interpretation. Recent studies by Bastia and co-workers (53) have demonstrated binding of IHF to a region of pSC101 near the origin of DNA replication. Binding of IHF to this segment results in enhanced bending of the DNA, and interference with the binding by mutations affecting the IHF consensus binding site in the replication origin region block IHF binding and abolish replication activity of the plasmid (53). It has been observed that bending of DNA is also associated with IHF binding to the *att* sites involved in λ integration (51, 55), in IHF binding to the outer ends of IS*1* and to a site in pBR322 (49), and in IHF binding to the replication enhancer region of phage f1 (24).

Mutations that can compensate for the lack of IHF in processes other than pSC101 replication have been described previously. A mutation in the *int* gene of λ (*int-h*) allows integration without IHF (34, 42). Derivatives of phage 21 and λ that no longer require IHF for phage DNA packaging have mutations in the terminase proteins of these phage (14, 15, 23); it is thought that IHF may aid proper binding of terminase to the phage *cos* sites, in which IHF-binding sites are located. Mutations in the phage f1 gene *II* protein allow replication in the absence of IHF. IHF normally binds to the AT-rich replication enhancer region of f1 (24). Mutations that allow expression of genes normally under IHF control in the absence of IHF have also been reported. The early genes of phage Mu are underexpressed in the absence of IHF, and an IHF-binding site is located in the promoter region for these genes (33); compensatory mutants that no longer require IHF have a mutation in the −10 region of the Mu P_e promoter (22).

Our data are consistent with the notion that IHF binding in the pSC101 origin region yields a molecular DNA conformation that allows the replication complex to be properly assembled (20, 53). Potentially, the structural alteration in the IHF-compensating mutant Rep protein could enable its interaction with other replication components in the absence of the DNA bending induced by IHF. Alternatively, the altered protein could itself influence the conformation of the plasmid DNA through its interactions.

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