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Integration Host Factor and Conjugative Transfer of the Antibiotic Resistance Plasmid R100

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Transfer of plasmid R100-1 was reduced 100-fold in the absence of integration host factor.

Integration host factor (IHF) of *Escherichia coli* is a heterodimeric DNA-binding protein (17) required for the chromosomal integration of bacteriophage lambda (21). The two chromosomal genes, *himA* (map position 37 min) and *hip* (map position 20 min), that encode the IHF subunits have been cloned and sequenced (6, 15). IHF has unexplained roles in the expression of the *ilvB* and *xyl* genes of *E. coli* (8). It functions in the regulation of bacteriophage Mu by controlling transcription of two converging promoters (11). IHF is also required for expression of the λ *c*II gene (10, 18). In this system it has been proposed that IHF affects translation because, in IHF mutants, short λ *c*II transcripts accumulate (13), similar to those seen when translation and transcription are uncoupled (19).

This communication shows that IHF is required for normal expression of another gene system in *E. coli*, namely, the transfer genes of the F-like plasmid R100-1. These R100-1 genes are contained in a 30-kilobase-long transfer operon (*tra*) and two separate single plasmid-specific transfer genes, *traM* and *traJ*, that are tandem to each other and immediately upstream from the *tra* operon. Expression of the *tra* operon requires the product of the plasmid gene *traJ* (5, 9), but the requirement of *traJ* product for *traM* expression is unclear (9, 16). *traJ* is a single, plasmid-specific positive control gene (9) which encodes a protein (1).

During probing of the effect of the R100 transfer control genes *finO* and *finP* on *traJ* transcripts, I found that *finO* caused accumulation of shortened *traJ* transcripts in the presence of *finP* just like those seen with λ *c*II and IHF mutants (Mol. Gen. Genet., in press). This similarity and the knowledge that IHF and genes identified as chromosomal transfer genes (*cpx*) both acted on one other system (8, 20) suggested that IHF might also affect transfer. Accordingly, R100-1 was introduced by conjugation from *E. coli* JC3272(R100-1) into an isogenic set of IHF testing strains kindly provided by H. Nash. [JC3272 is *his trp lys str gal lac* λ^r (λ def) (2).] The transconjugants were selected, maintained, and grown on glucose-M9 medium containing streptomycin (200 mg/liter) and spectinomycin (100 mg/liter). The IHF strains were N99 (*strR* su^- *galK* from W3102) and its derivatives HN678 (Δ*himA82* Tet^r [K5185]) and HN778 (Δ*hip-3* [*himD*] Cml^r [E444]). Transfer of R100-1 was measured as transfer of spectinomycin and tetracycline resistance from a freshly prepared set of such strains into VA8470, a nalidixic acid-resistant derivative of strain JC3272. Transconjugants were plated onto glucose-M9 medium containing (per liter) lysine (10 mg), tryptophan (10 mg), histidine (10 mg), tetracycline (25 mg), spectinomycin (100 mg), and nalidixic acid (40 mg). The conditions of mating were those of Finnegan and Willetts (4).

There was a 100- to 450-fold decrease in transfer of R100-1 in the absence of IHF (Table 1). Spot tests made with the male-specific phages f1, Qβ, and Mu2 showed that strain N99(R100-1) was sensitive to all three phages, whereas the *himA* and *hip* mutants were resistant. This confirmed that these IHF^- strains did not express transfer at a high frequency. (For reference, the table also shows the effect of the *finO* gene on transfer. Strains containing *finO* were likewise insensitive to the phages.)

Both of the IHF mutation strains contain chromosomal antibiotic resistance genes identical to genes residing on R100-1 (*cml* and *tet*). Although the male phage test argued against it, it remained possible that homologous recombination occurred between these genes and reduced apparent transfer by converting the plasmid to an Hfr strain. To test this possibility, the experiments were repeated with plasmid pWD6 in the same strains as given above. *amp* transfer was measured. (pWD6 is a spontaneous *spc cml sul mer* deletion mutant of pDU207 isolated in this laboratory. pDU207, an Amp^r Tet^s mutant of R100-1 originally described by Foster and Willetts [7], was kindly provided by N. Willetts.) No difference in results was found (Table 1). The conclusion was that the original transfer reduction was real and not an artifact of homologous recombination between chromosomal and plasmid antibiotic resistance genes.

In the mating protocol used here, stability of donor plasmids is tested by plating the donors on ML agar without antibiotics and then screening individual colonies for their antibiotic resistance patterns by the replica-plate technique. In the above-described crosses all donors were tested for spectinomycin and tetracycline resistance (or ampicillin resistance, for pWD6), and all were found resistant. In addition to this standard test, a test was performed to determine whether passage through IHF mutant strains altered the donor ability of R100-1. To do this, two colonies of each of the three VA8470(R100-1) transconjugants produced in the above-described crosses were purified by consecutive single-colony isolation and tested for donor ability of R100-1 to strain ED2149 [Δ(*nadA gal att*λ *bio*) *tsx* Δ*lacU124*] (2). Transconjugants from these secondary crosses were plated onto glucose-M9 medium containing (per liter) niacin (1 mg), biotin (0.1 mg), tetracycline (25 mg), and chloramphenicol (30 mg). Donors were counterselected by their amino acid requirements. An average value of 80 was found for the number of transconjugants per 100 donors for each of the strains. This indicated that the transferred plasmids retained their normal high transferability regardless of their passage through the IHF mutant strains.

The results (Table 1) establish that IHF is required for high-level transfer of R100-1. Examination of the R100-1

TABLE 1. Donor abilities of R100-type plasmids from *him* strains

Host strain	Plasmid donated[a]		
	R100	R100-1	pWD6
N99	0.21	36	54
HN678	8×10^{-5}	8×10^{-2}	0.34
HN778	1.5×10^{-4}	0.36	0.33

[a] Number of transconjugants per 100 donors at 37°C (30 min).

DNA sequence (3, 14) for the region containing *oriT*, *traM*, and the beginning of *traJ* shows six potential binding sites for IHF that have eight-of-nine base homology with the IHF consensus sequence (12). My colleagues and I are currently investigating the effects of IHF on transcripts from both *traM* and *traJ* cistrons and also the ability of IHF to protect the six potential sites against DNase I.

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