REVIEWS

Genetic Regulation of Plasmid Transfer

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Plasmids are extrachromosomal genetic elements which in bacterial cells occur as entities physically separated from the chromosome and, as such, are capable of self-maintenance and self-replication for an infinitely long time. They are ubiquitous, being present in bacteria of nearly all systematic groups, and are also found in cells of a number of eukaryotes. When residing in bacteria, plasmids impart to them important properties, such as drug resistance, toxin production, and a capacity to degrade organic compounds. One of the most remarkable properties of many of them (conjugative plasmids) is the ability to transfer genetic information from the host (donor) bacteria to other (recipient) bacteria. This property is determined by the genetic region *tra*, which, as has been established for the F plasmid, comprises more than 20 genes and controls the synthesis of proteins that make possible both the conjugation of donor to recipient bacteria and DNA transfer from donors to recipients. Most of these genes have been traced to the operon *traY* → *traZ* [31,32,34,36,37].

A positive regulator of the *traY* → *traZ* operon in the F plasmid is the *traJ* gene, whose product is required for the transcription of this operon. The *traJ* gene, however, is itself under the negative control of the OP system's genes that prevent its expression and, consequently, the expression of the *traY* → *traZ* operon [29,36]. Accordingly, as we noted earlier [17], the genetic regulation of F plasmid transfer may be described as a "two-story" one, being positive at one "story" and negative at the other. Yet there also exists a *traJ*-independent transfer that is not acted upon by a product of the OP system. Rather, as shown in several studies, the *traJ*-independent transfer is acted upon by products of other genetic systems, including *fin*Q, *fin*U, *fin*V, *fin*W, and *fin*C, identified in the genomes of self-repressed (rd) F-like and non-F-like plasmids [29,30,36].

Thus, as can be seen from the above, the regulation of plasmid transfer is effected by many genes. However, we still do not know how many genes are actually involved in regulating plasmid transfer, what the number of such genes is and how they interact in the genomes of separate plasmids or in those of plasmids that form complexes in bacteria, and what are the origins of regulatory genes. All these questions led us to undertake studies aimed at identifying other genes regulating *traJ*-independent plasmid transfer, unraveling the mechanisms of their action and interaction, and tracing their origins.

SEEKING AND IDENTIFYING *FIN* SYSTEMS IN RD-TYPE PLASMIDS

Before a search for other possibly existing *fin* systems could be started, it was necessary to have, as indicators, plasmids of the derepressed (drd) type sensitive to inhibitors of plasmid transfers determined by already identified *fin* systems. For this reason, drawing upon the available knowledge [29,30,36] of the genetic systems *fin*OP, *fin*Q,

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*fin*U, *fin*V, *fin*W, and *fin*C, which are capable of inhibiting *Tra* functions of the Flac plasmid, we started out by determining how sensitive to the inhibitors controlled by these systems are the F-like drd plasmids identified in our laboratory at different times [2,3,19-21]. As plasmids containing *fin* systems we used rd plasmids that determine inhibitors of known types, namely R100 (*fin*OP), R62 (*fin*Q), TP108 (*fin*Q), JR66a (*fin*U), R485 (*fin*V), R455 (*fin*W), and CloDF13cop3 (*fin*C) received from N. Willetts' Laboratory (Great Britain).

The sensitivity of these drd plasmids to inhibitors was estimated by determining the inhibitability of their *Tra* functions (i.e., the frequency of conjugational transfer and the activity of specific "sex" pili adsorbing pilus-specific phages from the F group) by rd plasmids.

The results obtained in estimating the sensitivities of the drd plasmids under study to known inhibitors of Flac plasmid transfer and in determining their ability (or inability) to inhibit the *Tra* functions of the Flac plasmid are presented in Table 1.

As shown in Table 1, unlike the "classic" F plasmid of the drd type, which is sensitive to inhibitors of all six known types (FinOP, FinQ, FinU, FinV, FinW, and FinC), the F-like drd plasmids from our collection proved to be sensitive at one and the same time to only one to five of the known transfer inhibitors. In other words, these F-like drd plasmids were each characterized by a more narrow range of sensitivity to transfer inhibitors than the F plasmid.

In evaluating our results, we came to the conclusion that some of the drd plasmids listed above could indeed be utilized as indicator test plasmids for the further identification of *fin* systems in the self-repressed plasmids. Accordingly, using the selected collection of F-like drd plasmids with known types of sensitivity to transfer inhibitors, we developed a special procedure to search for and identify *fin* systems of conjugative plasmids of the self-

TABLE 1. Sensitivity of drd Plasmid Transfer to Identified Inhibitors

drd Plasmid	Incompatibility group	Ability to inhibit Flac transfer	*fin* Systems to which drd plasmids are sensitive
pAP10−2::Tn9	FI	−	OP, Q, U, V
pAP11−2			OP, U, V, W
pAP11−2::Tn1	FI/FIV	+	OP, Q, U, V, W
pAP11−2::Tn5			OP, U, V
pAP11−2::Tn9	FI/FIV		OP, U, V, W
pAP18−1	FVII	−	V, W
pAP18−1::Tn5	FVII (partially)	−	V
pAP18−1::Tn9	FVII (partially)	+/−	V, W
pAP19−1::Tn1	FX	+/−	OP, Q, U, V, W
pAP19−1::Tn9	FVII	−	OP, U, V
pAP22−2			OP, V
pAP22−2::Tn1	FII/FIII/FIV (partially)	−	U, V
pAP22−2::Tn5	−		V
pAP38::Tn9	FVII	+	Q, U, V
pAP41::Tn9::Tn1721	−		OP, U, V
pAP42			OP, Q, U
pAP42::Tn1	FIX	+	OP, U, V
pAP42::Tn5		+	U, V, W
pAP42::Tn9	FIX	+	OP, U, V
pAP43::Tn5	FVIII	+	U, V, W
pAP53			V
pAP53::Tn5	FIII	−	V
pAP53::Tn9	FIII	−	V

Note. +(−) = ability (inability) to inhibit plasmid transfer; +/− = inhibitory effect is weak.

TABLE 2. Identification of *fin* Systems Regulating Transfer in Plasmids of the Self−Repressed Type

rd Plasmid	Ability to inhibit transfer of F-like drd plasmids (type of sensitivity to transfer inhibitors)					Probable type of rd plasmid inhibitor
	pAP53::Tn5 pAP53::Tn9 (U)	pAP22-2::Tn1 (U, V)	pAP18-1 pAP18-1::Tn9 (U, W)	pAP11-2::Tn5 pAP19-1::Tn9 (OP, U, V)	pAP10-2::Tn9 pAP11-2::Tn1 (OP, Q, U, V)	
pAP2	−	−	−	+	+	OP
pAP3	−	+	−	+	+	U
pAP7−1	−	+	−	+	+	U
pAP17−1::Tn9	−	+	−	+	+	U
pAP17−2	−	+	−	+	+	U
pAP18−1	+	+	ND	+	+	V
pAP28	−	−	−	+	+	OP
pAP30−1	−	+	−	+	+	U
pAP30−2	−	+	−	+	+	U
pAP31	−	+	−	+	+	U
pAP39::Tn9	−	+	−	+	+	U
pAP41::Tn1	+	ND	+	+	ND	V
pAP41::Tn9	+	+	+	ND	ND	V
pAP46	+	+	+	+	+	V

Note. +(−) = ability (inability) of the rd plasmid to inhibit *Tra* functions in the drd plasmid. ND = not determined.

repressible type (rd plasmids). The rd plasmids used had been identified by us previously in various naturally occurring *E. coli* populations [14,15].

As can be seen in Table 2, the genomes of these rd plasmids contained *fin* systems already described by other authors, the two systems encountered most frequently being *fin*U and *fin*V.

However, as we discovered in our further studies of the inhibitory potentials possessed by other rd plasmids [12], the inhibitory activity of some of them differs fundamentally from that of the rd plasmids in Table 2. The F-like plasmids pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, and pAP27 were found not to inhibit any of the Flac factor transfer functions. In appraising these findings, we supposed that the indicated plasmids fail to act on the F factor because their genomes contain *fin* genes that determine the synthesis of some other inhibitors, i.e., that the plasmids contain *fin* genes distinct from those previously described.

To check this hypothesis, we carried out experiments to determine the sensitivity of individual F-like drd plasmids to inhibitors controlled by the *fin* systems of pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, and pAP27. As shown in Table 3, the genomes of these plasmids indeed contain *fin* genes determining the synthesis of inhibitors that, while not inhibitory for the Flac plasmid, are active against F-like drd plasmids, inhibiting their transfer and piliation.

The *fin* systems identified in the plasmids pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, and pAP27 were designated as *fin*K, *fin*L, *fin*M, and *fin*N, respectively.

Having obtained these results, we undertook several experiments to establish genomic locations of the identified *fin* systems, including *fin*V, *fin*U, and *fin*N [4,9,11]. To this end, we used molecular cloning of different restriction DNA fragments from plasmids containing those systems in their genomes.

In order to localize the *fin*V system, the *EcoR*I and *Sal*I fragments of pAP18-1 were cloned within the vector plasmid pBR325. The subsequent complementation analysis using the resulting recombinant plasmids showed that the genetic *fin*V system of pAP18-1 occurs in its *Sal*I fragment f5 (3.9 MD). It was also found that this fragment contains both the genetic region controlling the surface exclusion of pAP18-1 (*sfx*II region) and some of the structural genes determining the transfer of this plasmid (*tra*2 region). An additional complementation analysis using a group of the identified F-like drd plasmids with mutational changes in the genetic region controlling the synthesis of a *Fin*V type inhibitor led us to conclude that this region has a complex (polygenic) organization [23]. The *finV* region consists of at least three distinct genes, and we designated these as A, B, and C.

To localize the *fin*U system, the *Hind*III fragments of pAP17-1::Tn9 were cloned, also within

the vector plasmid pBR325, and the *fin*U system was found to be located in the *Hind*III fragment f3 (8.2 MD) of AP17-1::Tn9.

The location of the *fin*N system was established by cloning the *BamH*I fragments of pAP27 within the vector plasmid pUC19: it occurs in the *BamH*I fragment f3 (5.8 MD) of this F-like rd plasmid.

EXPRESSIVITY OF *FIN* SYSTEMS

Previously, the expressivity of *fin* systems was studied only in cells of laboratory *E. coli* K-12 strains. Yet plasmids circulate in ecological niches among bacteria of different species, and it was therefore interesting to find out whether *fin* systems of the same plasmids occurring in different bacteria, or at least in bacteria of serologically typable and nontypable *E. coli* strains, are expressed to the same extent. Accordingly, we first constructed such strains by incorporating one inhibiting plasmid and one inhibitable plasmid (rd+drd) into *E. coli* cells, and compared the cells for efficiency of plasmid transfer.

As shown in Table 4, *fin* systems were more active in the cells of serologically nontypable *E. coli* strains. For example, the inhibition indices of drd-plasmid transfer were invariably higher for all tested rd plasmids contained in cells of the serologically nontypable strain AP132 than for cells of serologically typable strains. These differences were all statistically significant ($p<0.001$). This means that the expression of *fin* systems is subject to influence by the bacterial genome.

Similar results were obtained in our study of the expression of the genetic region *tra*, which is contained in the genomes of various F-like plasmids and is responsible for the formation of functional "sex" pili and of surface exclusion systems (*sfx* systems) in serologically typable *E. coli* strains [18].

Our finding that *fin* systems are subject to the influence of the bacterial genome and the reported evidence that chromosomal genes of *E. coli* K-12 donor cells influence the expression of functions performed by F plasmid *tra* genes [26-28] made us curious to find out which chromosomal segment of *E. coli* affects the expression of *fin* systems.

TABLE 3. *Fin* Effects of rd Plasmids pAP22−1::Tn1, pAP20::Tn9, pAP19−1::Tn9, and pAP27 on drd Plasmid Transfer Functions and Piliation

drd Plasmid	Sensitivity of drd plasmids to reference *fin* systems	*Fin* effect due to rd plasmid			
		pAP22-1::Tn1	pAP20::Tn9	pAP19-1::Tn9	pAP27
pAP10−2::Tn9	OP, Q, U, V	ND	ND	+	ND
pAP11−2::Tn1	OP, Q, U, V, W	+	+	ND	ND
pAP11−2::Tn5	OP, U, V	−	+/−	−	+
pAP11−2::Tn9	OP, U, V, W	ND	ND	−	ND
pAP18−1	V, W	−	+	+	ND
pAP18−1::Tn5	V	+/−	+	+	−
pAP18−1::Tn9	V, W	−	+	−	ND
pAP19−1::Tn1	OP, Q, U, V, W	+/−	+/−	ND	ND
pAP19−1::Tn9	OP, U, V	ND	ND	+	ND
pAP22−2::Tn1	U, V	−	−	ND	ND
pAP22−2::Tn5	V	−	+	+	−
pAP38::Tn9	Q, U, V	ND	ND	−	ND
pAP39::Tn9::Tn5	ND	−	−	−	+/−
pAP41::Tn1721	ND	+	+	−	ND
pAP41::Tn9::Tn1721	OP, U, V	+	+	−	ND
pAP42::Tn1	OP, U, V	−	+/−	ND	ND
pAP42::Tn5	U, V, W	−	−	+	+/−
pAP42::Tn9	OP, U, V	ND	ND	+	ND
pAP53::Tn5	V	+	+	+	+/−
pAP53::Tn9	V	ND	ND	−	ND

Note. + = *Fin* effect for plasmid transfer and piliation; +/− = *Fin* effect for plasmid transfer; − = no *Fin* effect. ND = not determined.

Our experiments with prototrophic and auxotrophic *E. coli* K-12 strains [5] revealed a clear-cut association between the genetic features of individual strains and the expressivity of the *fin*V system contained in the F-like rd plasmid pAP18-1. Our subsequent genetic analysis led us to conclude that the chromosome of *E. coli* K-12 cells has a genetic region essential for the normal expression of the plasmid system *fin*V; this region was designated as *tis* (*transfer inhibition stimulation*). As a study of genetic recombinants produced by crossing *Tis*$^+$ and *Tis*$^-$ cells showed, the *tis* region is linked to the chromosomal segment controlling the synthesis of threonine and leucine. This indicates that the chromosomal segment Thr-Leu plays a major role in the expression of the *fin*V system.

INTERACTION OF *FIN* SYSTEMS

Prior to our investigations reviewed here, the objects of studies on the regulation of genetic transfer were bacterial cells that contained only one rd plasmid carrying one of the *fin* systems. However, bacteria of naturally occurring strains contain, as a rule, plasmid complexes made up of two or more plasmids in one bacterial cell [1,7,8,16, 25,33,35], each of which can probably have its own *fin* system. This raises the question of how one *fin* system may act on or interact with another in a bacterial cell carrying more than one plasmid, each having its own systems of plasmid transfer.

Before addressing this question, we investigated a natural plasmid complex discovered in cells of an opportunistic *E. coli* strain and consisting of the F-like plasmid pAP18-1 (Tc, Col V) and the N-like plasmid pAP18-2 (Sm). Unlike pAP18-1, which codes for the synthesis of the *Fin*V inhibitor acting upon the *Tra* functions of the Flac plasmid, the rd plasmid pAP18-2 was found not to influence conjugative properties of the Flac plasmid and to contain, possibly for this reason, a different *fin* system in its genome. On the other hand, pAP18-2 is sensitive to the FinV inhibitor of pAP18-1, but not *vice versa*. These findings indicate that the plasmids of the complex are not indifferent to each other [6].

In further experiments, we constructed a number of artificial diplasmid complexes from plasmids containing *fin*V or *fin*U systems, and then examined their interaction in inhibiting the transfer of the Flac plasmid and of other F-like drd plasmids.

These experiments showed that if each of the two plasmids contains a *fin*V system, then the inhibitors controlled by these systems inhibit the transfer of the Flac and F-like drd plasmids to a much greater extent than in the case of each of the *fin*V systems acting separately. In contrast, no enhancement of the inhibitory effect was observed with diplasmid systems of the type *fin*U+*fin*U or *fin*U+*fin*V.

We also studied interactions of *fin* systems using cointegrative plasmids pAP42/pRSF2124 and pAP42/pUB781 constructed on the basis of the F-like transfer factor pAP42 (*fin*U) and nonconjugative plasmids pRSF2124 and pUB781 possessing *fin*V systems [13]. As these experiments showed, the genetic systems *fin*U and *fin*V incorporated into the genomes of cointegrative plasmids act in an additive manner, so that the effects of the composite inhibitor synthesized under the control of both these systems is much greater than the effects resulting from each of them separately.

Assessing the results presented above, we may conclude that the two *fin*V systems belonging to different plasmids in plasmid complexes and the *fin*U and *fin*V systems present in the genome of a single cointegrative structure exert an additive inhibitory effect, i.e., they interact. Why the *fin*V and *fin*U systems belonging to different plasmids in a complex fail to act in an additive manner is difficult to explain at the present time.

FIN SYSTEMS AND TRANSPOSABLE GENETIC ELEMENTS

Drawing on the reported evidence regarding the ability of transposable genetic elements (transposons) to induce mutations, i.e., to act as mutagens, we tested transposons for their effects on *fin* systems, taking into account the functional specificity of these elements. The results, summarized in Table 1, show that incorporation of various transposons into the genomes of F-like plasmids may result not only in derepression of the plasmids, because of the inactivation of their *fin* gene (plasmids pAP10-2::Tn9 and pAP41::Tn9::Tn1721), but also in their altered sensitivity to inhibitors of *Tra* functions [10,22].

Analysis of restriction maps for the drd plasmid pAP18-1 and its transposon-containing analogs [24] strongly suggests that such alterations may be associated with certain genomic rearrangements in this plasmid, caused by the incorporation of transposons in its genome. Moreover, insertion of the Tn5 transposon into the *EcoR*I fragment f8 (1.3 MD) of pAP18-1 led to the emergence of a new fragment, f4 (5.3 MD), and the insertion of Tn5 into the *Sal*I fragment f1, to the formation of two other fragments - f2 (11.5 MD) and f4 (8.3 MD). As regards Tn9, this transposon appears to

TABLE 4. Ability of rd Plasmids to Inhibit the Conjugational Transfer of F−like drd Plasmids in Cells Serologically Typable and Those Not Typable by *E. coli*

Composition of diplasmid transconjugates		Strain of host bacteria		Index of drd plasmid transfer inhibition
rd plasmid (type of transfer inhibitor)	drd plasmid (type of sensitivity to transfer inhibitors)	designation	serogroup	
pAP3 (U)	pAP11−2::Tn5 (OP, U, V)	AP15−3	O106	52±1
	AP70−3	AP58−3	O147	(26±2)×10
	AP132(K12)	O128	(88±3)×10	
		R form	(25±1)×100	
pAP17−2 (U)	pAP22−2::Tn1 (U, V)	AP15−3	O106	0.7±0.06
	AP70−3	AP58−3	O147	33±2
	AP132(K12)	O128	53±3	
		R form	(21±1)×1000	
pAP18−1 (V)	pAP53::Tn5 (V)	AP15−3	O106	54±5
	AP70−3	AP58−3	O147	(13±2)×10
	AP132(K12)	O128	(12±0.9)×100	
		R form	(43±3)×100	
pAP30−2 (U)	pAP11−2::Tn5 (OP, U, V)	AP15−3	O106	26±2
	AP70−3	AP58−3	O147	38±3
	AP132(K12)	O128	43±4	
		R form	(12±0.9)×100	

have been incorporated into the *EcoR*I fragment f6 (3 MD), which resulted in the emergence of two more fragments, f6 (2.6 MD) and f8 (2 MD), while the only result from the incorporation of Tn9 into the *Sal*I fragment f1 (15.8 MD) was an increase in the size of this fragment (to 17.4 MD). Restriction analysis of pAP42::Tn1 DNA revealed Tn1-induced structural changes in the genome of this plasmid, as compared with the DNA of the original plasmid pAP42. In particular, deletion of the *Hind*III fragment f7 (2 MD) present in the pAP42 DNA was detected, as was the appearance of a new *Hind*III fragment, f3 (4.9 MD), which was not present on the DNA electrophoregram of the original pAP42 plasmid.

Structural changes in DNA attributable to transposon insertions were also detected in the plasmids pAP22-2, pAP22-2::Tn1, and pAP22::Tn5. In particular, pAP22-2::Tn1 was found to differ from the original plasmid pA22-2 only in having a new *Sal*I fragment, f3 (9.0 MD); more significant differences were found for pAP22-2::Tn5.

The above findings demonstrate the possibility of modifying plasmid transfer-regulating genetic systems by inserting transposable genetic elements into plasmid genomes.

In light of the results of our experiments and the data reported in the literature, we may conclude that the most complex level (or "story") in plasmid transfer regulation appears to be the one that involves the regulation of *traJ*-independent transfer and which is characterized by an extraordinary diversity of genetic structures determining the regulation. Suffice it to say that at least 10 *fin* genes are active at that level, but the actual number of these gene systems may be even larger.

The question arises, why are the genes involved in the regulation of plasmid transfer so diverse? The mutations identified in the *fin*V system and the discovered ability of transposons to bring about genomic rearrangements in plasmids affecting their *fin* systems to the extent of inactivating them suggest that one explanation for the diversity of *fin* systems is their mutagenesis, whereby one *fin* system converts to another or *fin* systems arise *de novo*. Possibly, mutagenic factors inducing *fin* gene mutations under natural conditions are transposons.

The demonstrated additive effects of gene products which inhibit plasmid transfer and which are controlled by two *fin*V systems (by two plasmids) simultaneously indicate that these systems interact. However, the interaction between *fin* systems of different types probably depends on the *cis-trans* position of these systems in the genomes containing them. If plasmids are contained in a complex, then the burden of regulating the *traJ*-independent plasmid transfer is likely to fall on the *fin* system of one of the plasmids. If, on the other hand, two plasmids have formed a cointegrate, then their *fin* systems act in an additive manner even if they are distinct.

Consideration of the better known examples of gene expression regulation in prokaryotes and eukaryotes warrants the statement that neither of

these have been shown to possess a structural gene or a group of such genes whose expression is regulated by so many regulatory genes as in the case of plasmid *tra* genes. Recognition of this fact inevitably leads to the conclusion that the complexity of the regulation of expression of genes controlling particular traits does not depend on the level of organization of the organisms to which the genes belong. However, no satisfactory explanation can be offered for this phenomenon as yet.

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