Comparative Biology of IncQ and IncQ-Like Plasmids

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INTRODUCTION

Plasmids of the Escherichia coli incompatibility group Q (IncQ) and related IncQ-like plasmids are characterized by their relatively small size, their ability to be mobilized by several self-transmissible plasmids, and their broad host range (25). This combination of characteristics has resulted in plasmids of this type being highly promiscuous. As a consequence, IncQ family plasmids are found in a variety of very different environments. Since there are very few families of truly broad-host-range, highly mobilizable plasmids, plasmids of the IncQ family are good candidates for research into fundamental aspects of plasmid biology. The importance of these studies is increased because IncQ plasmids have been used as a major source of broad-host-range replicons in the development of genetic systems for a wide range of bacteria (5, 85).

As will be described in this review, the promiscuous nature of plasmids of the IncQ family has resulted in the acquisition of these plasmids by microorganisms growing in a number of very different environments. The effect of this is that there appear to be several evolutionary lineages of these plasmids, making the study of these plasmids interesting from the points of view of evolutionary plasmid molecular biology and the ecological distribution of plasmids. The replication, regulation, and mobilization functions of some of the prototypic IncQ plasmids have been extensively studied, and good reviews on these aspects are available (19, 25, 59, 76). The aim of this review is not to repeat material that has been reviewed elsewhere but, rather, to compare the IncQ and IncQ-like plasmids in the light of several recently discovered plasmids which belong to the IncQ family.

ECOLOGICAL DISTRIBUTION AND DIVERSITY

The most extensively studied plasmids of the IncQ family are the IncQ plasmids RSF1010, R1162, and R300B, which were isolated independently from E. coli (32), Pseudomonas aeruginosa (12, 53), and Salmonella enterica serovar Typhimurium, respectively (6). Although originating from different hosts, these plasmids each contain streptomycin and sulfonamide resistance genes and appear to be nearly identical. During investigations into plasmids containing antibiotic resistance genes from E. coli, other enterobacteria, and a variety of other bacteria of medical importance, several IncQ plasmids which contain additional or different antibiotic resistance genes to RSF1010 have been isolated (94). Not all of these plasmids...
have been sequenced completely, but in many cases it was shown that they have replicons which are incompatible with IncQ plasmids. Some characteristics of these plasmids are summarized in Table 1. Initial discoveries of IncQ and IncQ-like plasmids were restricted mainly to bacteria encountered in a medical context.

### Screening of Nonmedical Environments for IncQ-Like Plasmids

A study of the detection of IncQ plasmids in bacteria by using PCR amplification and a set of three pairs of IncQ-specific primers indicated that IncQ plasmids were prevalent in total DNA isolated from a variety of samples. These included soils from Germany and Holland and pig manure slurries from Germany (30). Although the presence of IncQ plasmids would have been detected in this investigation, the specific primers used would not have detected plasmids which are related to but different from IncQ plasmids. In another study, antibiotic resistance plasmids were captured in mating experiments in which the bacteria present in pig manure slurry were used as plasmid donors and selectable E. coli and Pseudomonas putida strains were used as recipients (86). From the sequence of plasmid pIE1107, which is IncQ like but different from IncQ plasmids, five sets of primers were designed from regions where plasmids RSF1010 and pIE1107 have conserved nucleotide sequence identity as well as from regions where they are different. These primers were used to detect whether IncQ and IncQ-like plasmids had been captured in the recipient bacteria.

### IncQ-Like Plasmids

A high prevalence of the two groups of IncQ and IncQ-like plasmids (prevalence approximately equal to IncP plasmids) was found, and furthermore a large amount of diversity in genotypic and phenotypic properties was discovered (30, 86). These workers suggested that plasmids with replicons which were incompatible with RSF1010 should be called IncQα and those with compatible replicons, IncQβ.

In contrast to the above studies, other investigators failed to detect IncQ plasmids in DNA hybridization experiments when using bacteria cultured from marine sediment microbial communities (88). Several broad-host-range plasmids were isolated, but none were of the IncQ type (89). Although IncQ plasmids themselves were not detected, this study does not eliminate the possibility that other less highly conserved members of the IncQ plasmid family may have been present. The exact region of the 357-bp IncQ plasmid probe was not given (88), and, depending on conservation of this region among plasmids of the IncQ family and the stringency of the hybridization and washing reactions, IncQ-like members of the IncQ plasmid family may not have been detected by these hybridization experiments.

### Types of IncQ-Like Plasmids

Besides the rather limited range of environments that have been screened for the presence of IncQ plasmids, several IncQ-like plasmids have been discovered serendipitously. Plasmid donors and selectable E. coli and Pseudomonas putida Typhimurium (United Kingdom)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Compatible with IncQ</th>
<th>Size (bp)</th>
<th>Source from which isolated</th>
<th>No. of copies per chromosome</th>
<th>Genes and ORFs in addition to backbone</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSF1010</td>
<td>No</td>
<td>8,684</td>
<td>E. coli typhimurium</td>
<td>12</td>
<td>sulII, strAB</td>
<td>32</td>
</tr>
<tr>
<td>R1162</td>
<td>No</td>
<td></td>
<td>P. aeruginosa</td>
<td>11</td>
<td>Sm, Su</td>
<td>12, 53</td>
</tr>
<tr>
<td>R300B</td>
<td>No</td>
<td></td>
<td>S. enterica serovar Typhimurium (United Kingdom)</td>
<td>11</td>
<td>Sm, Su</td>
<td>6</td>
</tr>
<tr>
<td>R678</td>
<td>No</td>
<td>±14,000</td>
<td>E. coli Dublin (Denmark)</td>
<td>11</td>
<td>Sm, Su</td>
<td>6</td>
</tr>
<tr>
<td>R684</td>
<td>No</td>
<td>±9,500</td>
<td>Proteus mirabilis (Germany)</td>
<td>11</td>
<td>Sm, Su</td>
<td>6</td>
</tr>
<tr>
<td>PB165</td>
<td>NR</td>
<td>±11,900</td>
<td>E. coli (United Kingdom)</td>
<td>9</td>
<td>Sm, Su</td>
<td>6</td>
</tr>
<tr>
<td>p89S</td>
<td>No</td>
<td>±8,180</td>
<td>E. coli (clinical)</td>
<td>NR</td>
<td>Su</td>
<td>75</td>
</tr>
<tr>
<td>pFM202</td>
<td>Yes</td>
<td>±7,100</td>
<td>Neisseria gonorrhoeae (Spain)</td>
<td>NR</td>
<td>Ap</td>
<td>73</td>
</tr>
<tr>
<td>pFM739</td>
<td>No</td>
<td>±9,450</td>
<td>Neisseria sicca (Spain)</td>
<td>NR</td>
<td>Ap, Sm, Su</td>
<td>74</td>
</tr>
<tr>
<td>pHD148</td>
<td>NR</td>
<td>±7,500</td>
<td>Haemophilus ducreyi</td>
<td>NR</td>
<td>Sm</td>
<td>1</td>
</tr>
<tr>
<td>pHDS.1</td>
<td>NR</td>
<td>±8,100</td>
<td>Actinobacillus pleuropneumoniae</td>
<td>NR</td>
<td>Sm, Su</td>
<td>98</td>
</tr>
<tr>
<td>pAZ1</td>
<td>NR</td>
<td>±8,000</td>
<td>S. enterica serovar Typhimurium type 179</td>
<td>NR</td>
<td>Su, Tp (DHFR type III)</td>
<td>24</td>
</tr>
<tr>
<td>pIE639</td>
<td>No</td>
<td>±11,100</td>
<td>E. coli O20:H</td>
<td>±12</td>
<td>aph(3’)-Id, sat3, strAB, sulII</td>
<td>94</td>
</tr>
<tr>
<td>pIE723</td>
<td>No</td>
<td>±9,500</td>
<td>E. coli O147:K88</td>
<td>±12</td>
<td>ant(2’)-Ia, strAB, sulII</td>
<td>94</td>
</tr>
<tr>
<td>pIE1107</td>
<td>No</td>
<td>8,520</td>
<td>Piggery manure</td>
<td>±12</td>
<td>aph(3’)-IId, sat3, strAB</td>
<td>86</td>
</tr>
<tr>
<td>pIE1115</td>
<td>No</td>
<td>10,687</td>
<td>Piggery manure</td>
<td>±12</td>
<td>linB-like, strAB, sulII</td>
<td>86</td>
</tr>
<tr>
<td>pIE1120</td>
<td>No</td>
<td>±9,100</td>
<td>Piggery manure</td>
<td>±12</td>
<td>tetA(Y), strAB</td>
<td>86</td>
</tr>
<tr>
<td>pIE1130</td>
<td>No</td>
<td>10,687</td>
<td>Piggery manure</td>
<td>±12</td>
<td>aph(3’)-I, catIII, strAB, sulII</td>
<td>86</td>
</tr>
<tr>
<td>pDN1</td>
<td>Yes</td>
<td>5,112</td>
<td>Dichelobacter nodosus</td>
<td>NR</td>
<td>None</td>
<td>96</td>
</tr>
<tr>
<td>pTF-FC2</td>
<td>Yes</td>
<td>12,190</td>
<td>A. ferrooxidans</td>
<td>12–15</td>
<td>grx, merR-like, ORF43, mpPct, tnpA</td>
<td>17</td>
</tr>
<tr>
<td>pTC-F14</td>
<td>No</td>
<td>±14,000</td>
<td>A. caldus</td>
<td>12–16</td>
<td>Unknown</td>
<td>27</td>
</tr>
</tbody>
</table>

a The exact gene is given where known; otherwise, the type of antibiotic resistance expressed is given. Ap, ampicillin; Cl, clindamycin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Lm, lincomycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; To, tobramycin; Tp, trimethoprim. Cl/Lm conferred by linB; Cm conferred by catIII; Km/Nm/Tb conferred by ant(2’)-Ia; Km/Nm conferred by aph(3’)-IId; streptothricin resistance conferred by sat3; Sm conferred by strAB; Su conferred by sulI; Tp conferred by tetA(Y).

b Gene incomplete or truncated.

c Unidirectionally (asymmetrically) incompatible with RSF1010.

d Two oriV-like regions; the nonfunctional oriV is incompatible with IncQ.

e E. Tietze, unpublished estimate.

TABLE 1. Characteristics of some naturally isolated IncQ and IncQ-like plasmids

Reference(s)

The references cited in the table are as follows:

1. Screen for IncQ plasmids in DNA hybridization experiments using bacteria cultured from marine sediment microbial communities.
2. Evaluate the prevalence of IncQ and IncQ-like plasmids in different environments.
3. Confirm the genotypic and phenotypic properties of IncQ-like plasmids found serendipitously.
4. Investigate the compatibility of IncQ-like plasmids with IncQ and IncP plasmids.
5. Assess the diversity of IncQ-like plasmids in different bacterial species.
6. Compare the characteristics of IncQ-like plasmids isolated from medical and nonmedical environments.

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mid pdN1 was isolated from the anaerobic bacterium, *Dichelobacter nodosus*, which is the principal causative agent of ovine footrot (96). This (5.1-kb) plasmid is closely related to pIE1107 but contains no antibiotic resistance genes and no other accessory genes. In contrast to the plasmids isolated from medical and animal environments, two IncQ-like plasmids have been isolated from bacteria present in inorganic biomining environments. Plasmid pTF-FC2 was isolated from a strain of *Acidithiobacillus ferrooxidans* (previously *Thiobacillus ferrooxidans*) that was being prepared as an inoculum for a biooxidation plant to treat a gold-bearing arsenopyrite ore concentrate (68). *A. ferrooxidans* is an acidophilic (optimum pH, 1.5 to 2.5) iron- and sulfur-oxidizing bacterium which is obligately chemolithoautotrophic and has a very low tolerance for organic matter (40). Approximately 15 years later, plasmid pTF-F14 was isolated (27) from a strain of a related bacterium, the moderately thermophilic (50°C), acidophilic (pH 1.3 to 2.0), chemolithoautotrophic, sulfur-oxidizing bacterium, *Acidithio-bacillus caldus* (previously *Thiobacillus caldus*) (34). This *A. caldus* strain f was isolated from a pilot biooxidation plant being used to treat a nickel-containing ore concentrate. The nucleotide sequences of six IncQ and IncQ-like plasmids have been published. These are plasmids RSF1010, pIE1107, pIE1115, pIE1130 (last two also captured from piggery manure [86]), pDN1, and pTF-FC2. In addition plasmid, pTC-F14 (27) has been partially sequenced. The characteristics of a selection of IncQ-like plasmids are shown in Table 1, and complete or partial genetic maps of these plasmids are given in Fig. 1. Based on available data from all sequenced members of IncQ-like plasmids, it is evident that the primer sets used would not have enabled the detection of IncQ-like plasmids such as pTF-FC2 or pTC-F14. The prevalence and variety of the IncQ family of plasmids in many environments is therefore unknown, and it is likely that many more types will be discovered.

**Host Range**

As described above, one of the most remarkable features of IncQ plasmids is their exceptionally broad host range. The list
of organisms in which IncQ plasmids and IncQ plasmid-based cloning vectors have been reported to replicate is shown in Table 2. Although possibly not complete, the list includes a wide variety of hosts belonging to the kingdom Bacteria (including gram-negative bacteria, gram-positive bacteria, and cyanobacteria) but no members belonging to the kingdoms Archaea or Eucarya. It has been suggested that the broad-host-range properties of the IncQ plasmids occur because the plasmid possesses many of the genes required for its own replication and this allows it to be more independent of the host replication machinery than are most other plasmids. RSF1010 has genes encoding its own plasmid-specific DNA-binding protein, a helicase, and a primase (19, 80). Scherzinger et al. (77) reported that besides these three essential plasmid-encoded proteins, E. coli host-encoded single-strand-binding protein, an $\alpha$ subunit of the DNA polymerase III holoenzyme were required for RSF1010 replication in vitro. Plasmid replication was independent of the host RNA polymerase, initiator DnaA protein and primosome, DnaB, DnaC, DnaG, and DnaT proteins. This result was consistent with their in vivo results.

Investigations into the host range of the IncQ-like plasmids are less extensive. However, plasmids pIE1115 and pIE1130 can be mobilized to and replicated in (albeit with varying stability) Pseudomonas putida, Acinetobacter sp. strain BD14, Ralstonia eutropha, and Agrobacterium tumefaciens (86). Likewise, studies of the ability of pTF-FC2 to replicate in hosts other than A. ferrooxidans indicate that the host range of this IncQ-like plasmid may be as wide as that of RSF1010. Work in the author’s laboratory has shown that pTF-FC2 is able to replicate in A. tumefaciens, E. coli, Klebsiella pneumoniae, P. aerugi- no$\text{osa}$, P. putida, S. enterica serovar Typhimurium, Sinorhizobium meliloti, and Thiobacillus novellus (67). Other workers have reported that plasmid pTF-FC2-based vectors are able to replicate in Acidiphilus facilis, Myxococcus xanthus (67), and Pseudomonas fluorescens (37). It is possible that IncQ and IncQ-like plasmids are unable to replicate in certain bacteria but that these negative results have not been reported. For example, attempts to transfer pTF-FC2 into the strict anaerobe Bacteroides fragilis using a clindamycin resistance gene as the selectable marker were unsuccessful (D. E. Rawlings, unpublished data).

### COMPARISON OF PLASMID BACKBONE STRUCTURES

The concept of a plasmid backbone includes genes and sites required for typical plasmid-associated functions such replication, conjugation, and stability (61, 92, 97). The determination of the extent of this backbone may be assisted by comparing features shared by most members of a plasmid family. Plasmids of the IncQ family have a backbone structure consisting of a region required for plasmid mobilization and a region required for replication and maintenance. Not all members have a plasmid stability system, but in the members which do, this system is integrated within the replicon with elements which are essential for replication on either side (Fig. 1). The stability system has therefore also been considered to be part of the plasmid backbone.

**Replicon Structure and Mechanism of Replication**

A model of the mechanism for the replication of the IncQ plasmids has been reviewed in detail, and only a summary will be presented here (25, 76). The IncQ replicon consists of three genes, repA, repB, and repC, and an oriV region (80). The oriV region contains 3.5 20-bp iterons with 2-bp nucleotide spacers. The iterons exert incompatibility and serve as binding sites for the site-specific DNA-binding protein, RepC (35, 48, 65). Binding of RepC is essential for replication and is thought to introduce conformational changes leading to DNA unwinding in the adjacent AT-rich region (35, 42). This RepC-induced DNA melting serves as an entry site for RepA, a plasmid-specific helicase which unwinds the DNA in the flanking regions. One of these flanking regions contains a large inverted repeat which has two plasmid-specific, single-stranded DNA initiation sites, ssiA and ssiB (35, 39, 49). The ssiA and ssiB sites initiate the priming of single-stranded DNA synthesis on opposite strands in the leftward and rightward directions, respectively (54). None of the conserved amino acid sequence motifs associated with the four helicase superfamilies can be easily identified in the RepA helicases of the IncQ plasmid family (33). Initiation of DNA synthesis is dependent on the specific plasmid-encoded primase, RepB (RepB') (38). The RepB primase occurs in two forms, a 78-kDa MobA-RepB fusion protein (Fig. 1) and a 36-kDa RepB' form, which is translated from an initiation codon downstream of and in the same reading frame as the fusion protein (76, 80). The model for plasmid replication predicts that it takes place by a strand displacement mechanism.

The overall layout of the genes and many of the other features of the members of the IncQ-like plasmid family are very similar. All IncQ-like plasmids have genes which encode or-

### TABLE 2. List illustrating the range of bacteria in which IncQ plasmids or IncQ-derived plasmid vectors can replicate

<table>
<thead>
<tr>
<th>Host</th>
<th>Plasmid or vector replicon</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp.</td>
<td>pIE1120</td>
<td>85</td>
</tr>
<tr>
<td>Acidithiobacillus ferrooxidans</td>
<td>pSUP106</td>
<td>66</td>
</tr>
<tr>
<td>Acidithiobacillus ferrooxidans</td>
<td>pKMZ1, pKT230</td>
<td>44, 63</td>
</tr>
<tr>
<td>Actinobacillus actinomycetemcomitans</td>
<td>pBK1</td>
<td>28</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td>RSF1010, pIE1120</td>
<td>8, 85</td>
</tr>
<tr>
<td>Alcaligenes sp. strain BR60</td>
<td>pMMB66HE</td>
<td>56</td>
</tr>
<tr>
<td>Arthrobacter sp.</td>
<td>pKT240, pML10</td>
<td>52</td>
</tr>
<tr>
<td>Bartonella henselae</td>
<td>IncQ vectors</td>
<td>18</td>
</tr>
<tr>
<td>Bdellovibrio bacteriovorus</td>
<td>IncQ vectors</td>
<td>16</td>
</tr>
<tr>
<td>Brevibacterium methylicum</td>
<td>RSF1010</td>
<td>57</td>
</tr>
<tr>
<td>Burkholderia pseudomallei</td>
<td>pKT230</td>
<td>50</td>
</tr>
<tr>
<td>Caulobacter crescentus</td>
<td>RSF1010</td>
<td>9</td>
</tr>
<tr>
<td>Chlorobium tepidum</td>
<td>pDSK519, pGSS33</td>
<td>95</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>RSF1010 derivatives</td>
<td>4</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>RSF1010 vectors</td>
<td>84</td>
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<td>Francisella novicida</td>
<td>RSF1010 derivative</td>
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</tr>
<tr>
<td>Haemophilus parainfluenzae</td>
<td>RSF1010 derivatives</td>
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</tr>
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<td>Legionella pneumophila</td>
<td>RSF1010</td>
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<tr>
<td>Mycobacterium smegmatis</td>
<td>RSF1010</td>
<td>29</td>
</tr>
<tr>
<td>Pseudoonaebuena sp.</td>
<td>pSUP1021, pKT230</td>
<td>90</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>pIE1120</td>
<td>85</td>
</tr>
<tr>
<td>Raistonia europha</td>
<td>pIE1120</td>
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<td>Streptomyces lividans</td>
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<td>29</td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td>pSUP1021, pKT230</td>
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</tr>
<tr>
<td>Synechocystis sp.</td>
<td>pSUP1021</td>
<td>90</td>
</tr>
</tbody>
</table>
thologs of the IncQ RepA helicase, the RepB' primase, and the RepC iteron-specific DNA-binding protein (Fig. 1).

**Comparison of replication proteins.** Amino acid sequence alignments of the three Rep proteins have indicated that based on protein sequence comparisons, the IncQ plasmid family may be divided into two major groups (Fig. 2). Plasmids pTF-FC2 and pTF-F14 comprise one group, and plasmids RSF1010, pIE1107, pIE1115, pIE1130, and pDN1 make up the other. The RepC DNA-binding proteins are the most highly conserved of the Rep proteins, with 62% amino acid sequence identity between the different groups and >74% identity between members of the same group. Based on the amount of sequence variation between groups, the RepA helicases are intermediately conserved, with 44% amino acid identity between groups and >81% identity within groups. At only 15% amino acid sequence identity, the RepB' primases are poorly conserved between groups, but at >74% amino acid identity, they are fairly well conserved within groups.

Given the sequence similarity between Rep protein orthologs, it is of interest to know whether any of the Rep proteins can functionally complement mutants in any of the others. Experiments of this type have been carried out between the IncQ replicons of plasmid R300B and pTF-FC2. The oriV region of pTF-FC2 could be complemented in trans by its own replication proteins but not by those of R300B. Frameshift point mutations within the repA and repC genes of pTF-FC2 were constructed, and although these mutants could be complemented by homologous unmutated repA and repC genes present on R300B (21). By cloning the oriV region of pTF-FC2 into a priming-deficient M13 phage and testing for an increase in plaque size, it was shown that RepB' of pTF-FC2 acted as a primase on its own oriV (22) while the R300B repB gene was unable to prime the oriV of pTF-FC2 (Rawlings, unpublished). Together, these studies indicate that the RepA, RepB', and RepC proteins of the R300B were unable, individually or in combination, to substitute for the orthologous proteins of plasmid pTF-FC2. Plasmids pTF-FC2 and R300B belong to different IncQ-like family groups, and it is possible that complementation between the more similar Rep proteins within a plasmid group might occur. However, initial studies using the oriV regions of the fairly closely related plasmids pTF-FC2 and pTC-F14 showed that either set of replication proteins was able to complement only its own oriV (27).

**Origins of replication.** The location of oriV regions relative to the replication proteins differs between the IncQ and the IncQ-like plasmids. In the IncQ-like plasmids, pDN1, pIE1107, pIE1115, pIE1130, pTF-FC2, and pTC-F14, the functional oriV is situated immediately downstream of the repC gene (Fig. 1). In plasmids pTF-FC2 and pTC-F14, this association is so close that the oriV region begins within the open reading frame of the COOH terminus of the RepC protein. In contrast, in the IncQ plasmid RSF1010 there is a DNA fragment of about 3.65 kb containing the sulII, strA, and strB genes, which separates the oriV region of the IncQ plasmids from the repC gene. The physical linkage of repC and oriV is therefore not a requirement and does not appear to confer a competitive disadvantage since the IncQ plasmids are widely distributed, especially in medical environments. Plasmid pIE1107 is unusual in that it has two oriV-like regions. oriVa is almost identical in location and sequence to the oriV of the IncQ plasmids. There is, however, a 19-bp deletion in the 8-bp stem-loop region which follows the AT-rich region, and oriVa is nonfunctional for plasmid replication (93). Plasmids pIE1115 and pIE1130 also have duplicate oriVa regions similar to pIE1107 but without the 19-bp deletion.

One of the most distinguishing characteristics of the oriV regions of members of the IncQ plasmid family is the presence of iterons (directly repeated sequences) with a 22-bp unit length. In plasmids RSF1010, pDN1, pIE1107, pIE1115, and

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**FIG. 2.** Phylogenetic relationships between Rep proteins of the IncQ plasmid family. Percentages represent percent amino acid sequence identities.
pIE1130, the iterons are 20 bp long with 2-bp spacers, whereas in pTF-FC2 and pTC-F14 the iterons are 22-bp long without spacers. The number of iterons varies from three to five (Fig. 3), with 10 of the nucleotides within the direct repeats being conserved among all members of the family. A short distance from the direct repeats is a GC-rich region of 28 bp followed by an AT-rich region of 31 bp. It has been proposed that RepC-mediated DNA melting occurs at this AT-rich region (mentioned earlier), which allows access to the plasmid-encoded RepA helicase. Plasmid pTC-F14 is an exception in that no clear GC-rich region is apparent. Figure 4 shows an alignment of the direct-repeat GC-rich and AT-rich regions of five members of the IncQ plasmid family. The respective oriVa-like regions of pIE1107, pIE1115, and pIE1130 have been omitted because they have identical nucleotide sequences to RSF1010 within the region shown. There is 55% nucleotide sequence identity among all IncQ-type oriV regions within this region, with a 14- of 15-bp consensus sequence identity in the region immediately downstream of the AT-rich region. There is an abrupt decrease in the consensus nucleotide sequence identity in the repC-distal region adjacent to that shown in Fig. 4. The region where the oriV nucleotide sequence alignment is poor includes the region containing the IncQ plasmid ssiA and ssiB sites. None of the other members of the IncQ-like plasmid family have an extended inverted-repeat region (40-bp stem and 40-bp loop) identical to that present in the IncQ plasmids (48). Members of the IncQ-like plasmids have other sequences capable of forming stem-loop-like structures in the AT-rich and repC-distal oriV region (Fig. 3), but the biological activity of these has not been tested. An exception is pTF-FC2, where an exonuclease III-created deletion midway through the cirB stem-loop resulted in poor plasmid replication and a greatly reduced copy number (20).

**Incompatibility groups.** Incompatibility is the inability of coresident plasmids to be stably inherited in the absence of external selection (58). Replicon-associated incompatibility is believed to arise due to the inability of the cell to correct fluctuations in copy number between plasmids that have elements of their replication machinery in common. It has been established that the iterons of the IncQ plasmid family are themselves able to exert plasmid incompatibility (48, 65). Since the sequences of the iterons of plasmids of the IncQ family are related, it has been of interest to determine which members of the family are incompatible. All incompatibility testing has been carried out with *E. coli* host strains. Plasmids pIE1107 and RSF1010 were found to be incompatible due to the IncQ-identical iterons present in the nonfunctional oriVa-like region (93). If this region was deleted, the resulting oriVb-containing plasmid, pIE1108, was fully compatible with the IncQ plasmids. The more recently discovered plasmids pIE1115 and pIE1130 also have nonfunctional IncQ-identical iterons, which make them incompatible with RSF1010 (E. Tietze, unpublished data). If these are deleted, plasmid RSF1010 belongs to one incompatibility group, plasmids pIE1108, pIE1115, and

![Schematic diagram of the structural features within the active oriV and nonactive oriV-like regions of IncQ-like plasmids. oriVa and oriVb indicates duplicate oriV regions in the same plasmid. oriVa indicates the nonfunctional but incompatibility active oriV-like region. Arrows pointing in the same direction represent directly repeated sequences and arrows pointing toward each other inverted repeat sequences capable of forming stem-loop structures. Numbers above the arrows indicate the number of bases conserved in imperfectly conserved iterons, and #11/20 indicates a truncated 11bp iteron. A+T, on A+T-rich region; C+G, a G+C-rich region; ssiA and ssiB; single strand initiation sites. DNA synthesis proceeds in a single direction toward the right-hand side from ssiA and toward the left from ssiB. The sequence to the left of the highly conserved 14/15-bp region is more highly conserved than that to the right.](http://mmbr.asm.org/)

**FIG. 3.** Schematic diagram of the structural features within the active oriV and nonactive oriV-like regions of IncQ-like plasmids. oriVa and oriVb indicates duplicate oriV regions in the same plasmid. oriVa indicates the nonfunctional but incompatibility active oriV-like region. Arrows pointing in the same direction represent directly repeated sequences and arrows pointing toward each other inverted repeat sequences capable of forming stem-loop structures. Numbers above the arrows indicate the number of bases conserved in imperfectly conserved iterons, and #11/20 indicates a truncated 11bp iteron. A+T, on A+T-rich region; C+G, a G+C-rich region; ssiA and ssiB; single strand initiation sites. DNA synthesis proceeds in a single direction toward the right-hand side from ssiA and toward the left from ssiB. The sequence to the left of the highly conserved 14/15-bp region is more highly conserved than that to the right.
The copy number of several members of the IncQ plasmid family have been reported (25) (Table 1). It has been estimated that RSF1010 has a copy number of 10 to 12 per chromosome in *E. coli* and *P. aeruginosa* (76). This is approximately the same as the 12 to 15 copies (20) or 10 to 14 copies (87) per chromosome estimated for pTF-FC2 and the 12 to 16 copies estimated for pTC-F14 in both *E. coli* and *A. caldus*. Like other iteron-containing plasmids, the copy number of IncQ plasmids is affected by the number of functional replicon-associated iterons. Becker and Meyer (7) determined the effect of inserting extra iterons on the copy number of a derivative of plasmid R1162. With two additional iterons, the number of the IncQ replicons dropped from 10–12 to only 5–7 copies per chromosome, and with three or four extra iterons it dropped to only 4–6 copies. They found that the low-copy-number derivatives were lost in the absence of selection and suggested that because R1162 does not possess a plasmid partitioning or stability system, it requires a high copy number to ensure stable inheritance by random distribution. It was proposed that this requirement for a high plasmid copy number placed a substantial metabolic burden on the cell which could enhance the selection of low-copy-number variants (7). Low-copy-number plasmids may be easily lost from the population, which favors the acquisition of accessory genes, which require a high copy number to function. This may place a restriction on the type of accessory genes which can be accommodated by the plasmid. It has been observed that several IncQ and related plasmids do have a substantial variety of accessory genes (Table 1) (Tietze, unpublished) but that many of these do not have consensus promoters or ribosome-binding sites. These genes may require a high copy number to ensure a sufficient level of gene expression. Copy numbers of plasmids of the IncQ family are remarkably consistent, at between 10 to 16 per chromosome, in spite of the number of iterons varying from three (pTF-FC2) to five (pTC-F14). In the IncQ-like plasmids with more than three iterons, the additional iterons all vary slightly from the consensus three. This variation takes the form of an extra base, a missing base, or a deletion of one of the bases of the 2-bp spacer region. It has been shown that relatively minor changes in iterons reduce or eliminate their functionality (55), and it may be that the sometimes small changes in the iterons (above the basic three) are sufficient to reduce their biological activity to maintain the copy number of IncQ and IncQ-like plasmids within the 10 to 16 copy range. Although this speculation remains to be tested, indications from standardized plasmid isolations are that all of the pE-named plasmids have a copy number comparable to that of RSF1010 (Tietze, unpublished). It is possible that plasmids which have a toxin-antitoxin stability system (e.g., pTF-FC2 [see below]) would not have needed to depend on copy number to prevent plasmid loss and could have tolerated a lower
copy number than would plasmids where no stability system has been detected (e.g., RSF1010). However, toxin-antitoxin systems confer a relatively moderate (2.5- to 100-fold) increase in plasmid stability, and it is interesting that the “standard” copy number of plasmids of the IncQ family has been preserved irrespective of whether a stability system is present.

Mobilization Regions

Although none are self-transmissible, all plasmids of the IncQ family may be mobilized at high frequency in the presence of self-transmissible helper plasmids. Not all conjugative plasmids are equally effective at mobilizing IncQ or related plasmids, but conjugative plasmids of the E. coli IncPα (e.g., RK2/RP4/R68) and IncPβ (e.g., R751) groups (53, 69) are particularly efficient mobilizers of these plasmids. By using helper plasmids, the IncQ plasmids have been successfully mobilized to a large number of hosts including a wide range of gram-negative bacteria, several gram-positive bacteria including Arthrobacter spp. (52), Streptomyces lividans, and Mycobacterium smegmatis (29), cyanobacteria such as Synechococcus (43), and plant (13) and animal (99) cells. Likewise pTF-F2C has been mobilized from E. coli to Pseudomonas fluorescens (37) and from Agrobacterium tumefaciens to plant cells (Rawlings, unpublished). RSF1010 can be efficiently mobilized by several other conjugation systems. For example, for mobilization into plant cells (13) and between agrobacteria (8), the T-DNA transfer apparatus of A. tumefaciens was used. RSF1010 plasmids can also be efficiently mobilized by chromosomally located icm/dot macrophage-killing virulence system of Legionella pneumophila (81, 83). Furthermore, it has been shown that some components of a type IV secretion system of L. pneumophila can substitute for some components of the icm/dot system that are required for RSF1010 mobilization (82).

Unlike the uniform replicons of the global IncQ plasmid family, all of which have repA, repB, and repC genes as well as several conserved features of the oriV in common, the mobilization systems are of two distinct types. The IncQ plasmid RSF1010, as well as pEl1107, pEl1115, pEl1130, and pDN1, comprise one mobilization subgroup and have a three-Mob-protein “IncQ-type” system (Fig. 1) which has substantial similarity to the two-Mob-protein system of A. ferrooxidans plasmid pTF1 (23). In contrast, the IncQ-like plasmids pTF-F2C and pTc-F14 have a five-Mob-protein mobilization system (71) with a close resemblance to the TraI systems of the IncP plasmids and form a second subgroup.

IncQ-type systems. IncQ and other plasmids of that subgroup have a mobilization system which consists of MobA, MobB, and MobC proteins and an oriT-containing region. MobA is a multifunctional protein consisting of an N-terminal relaxase (78, 79) and a C-terminal DNA primase (36). The two domains appear to be able to function independently of each other. The C-terminal domain is synthesized from the same region and reading frame as the RepB protein, which is associated with vegetative plasmid replication. The relaxase activity is responsible for nicking the DNA at the oriT site, and the MobA primase activity is required for initiation of DNA replication that takes place during conjugal transfer. MobB and MobC serve as accessory proteins which enhance nic site cleavage and DNA transfer. MobB is synthesized from within the mobA gene but in a different reading frame, while MobC is transcribed divergently from a gene which lies on the opposite side of oriT (80) (Fig. 1). It has been proposed that MobB enhances relaxosome stability and nic site cleavage (64) while MobC helps unwind the DNA in the vicinity of the nic site, allowing easier access to the MobA relaxase (101). Interestingly, plasmid pTF1 appears to have only two mobilization proteins, MobL, which has an amino acid sequence related to the first 386 amino acids (aa) of the N-terminal relaxase region of MobA, and MobS, which is related to MobC (23). No equivalent of the accessory MobB protein appears to be present, and no open reading frames previously identified with DNA primase activity have been found on this 6,657-bp plasmid (47). A dendrogram showing the sequence relationship between the relaxase portion (assumed to be the N-terminal 400 aa) of the 709 aa MobA protein of RSF1010 is shown in Fig. 5. There are clearly three groups of MobA relaxases related to the IncQ plasmids. One group contains plasmids with replicons related to the IncQ plasmids as well as pAB6 and pSC101; a second group contains pTF1, pNAGR23, and the Ti plasmids; and a third group contains plasmids of gram-positive bacteria. There are few plasmids in the database containing proteins related to the IncQ MobC accessory proteins; MobS of pTF1 is the most clearly related.

IncP-related systems. The alternate mobilization system of the IncQ plasmid family consists of five proteins (MobA, MobB, MobC, MobD, and MobE) and an oriT-containing region (71). These proteins have low but clear amino acid sequence similarity to the TraI, TraJ, TraK, TraL, and TraM proteins of the TraI region of the IncP plasmids, respectively (71). This relationship between the mobilization region of this group of IncQ-like plasmids and the TraI region of the IncP plasmids also applies to the order of the genes and the divergent nature of their transcription (Fig. 6). In the IncP plasmids, TraL is a relaxase of between 732 aa (RP4) (61) and 747 aa (R751) (92), and 409 aa of the N-terminal region of the MobA protein of pTF-F2C has 26% sequence identity to the 458-aa N-terminal region of the RP4 TraI. TraJ is a DNA-binding protein that is thought to alter the local DNA structure and allow the TraL relaxase to access its binding site (60, 100). TraK is an accessory protein, also with DNA-binding activity, which is believed to wrap around a ±180-bp region of DNA near oriT, changing the superhelicity and further assisting TraL to access its target site (100, 102). The functions of TraL and TraM are unknown, although TraL has a Walker A/ATP/GTP-binding site (92).

Origins of transfer. Transfer origins play an essential role in the initiation of transfer-associated DNA replication and in the processing and transport of DNA from a donor to a recipient cell (46). Through comparison of a wide variety of transfer origins, five families of oriT sequences which show strong nucleotide conservation in the vicinity of the nic site have been identified (46, 100). The two- to three-Mob-protein IncQ-like plasmids belong to an oriT family which includes pTF1, pSC101, and the oriT of pTiC58. The IncQ plasmids are all highly conserved within this region, except that R1162 has a rare 1-bp mismatch compared with other members of the IncQ group (Fig. 7). The five-Mob-protein IncQ-like plasmids belong to a different oriT group, which includes the IncP plasmids.
RP4 and R751 and the left and right borders of the T-DNA of the Ti plasmids (Fig. 7).

ACCESSORY DNA

In addition to plasmid backbone DNA, most plasmids of the IncQ family have accessory DNA of some sort. The exception is plasmid pDN1, which appears to consist only of plasmid backbone. It is possible that plasmids without accessory genes are more common than recognized but that since these plasmids lack selectable markers they are not easily detected and therefore appear to be more rare than they actually are. As may be expected, the accessory DNA from plasmids isolated from medical or animal environments contain mainly antibiotic resistance genes, but these are also the markers for which most screening has been done. Although initial isolates of IncQ plasmids possessed mainly sulfonamide and streptomycin resistance genes, IncQ and IncQ-like plasmids may encode a wide variety of resistance genes (Table 1). Like many antibiotic resistance plasmids, acquisition of many of these genes has been as a result of transposon, insertion sequence, or integron activity. In RSF1010, the antibiotic resistance genes clearly have a different G/C content and codon usage compared with the rest of the plasmid backbone, which indicates that they have been acquired relatively recently (80). With pIE723, the aadB antibiotic resistance cassette has been inserted into a

![FIG. 5. Phylogenetic relationship between the MobA and MobC proteins of the IncQ-1-type plasmids and their comparison with related relaxases of mobilizable and self-transmissible plasmids. Since in the IncQ-1 plasmids the MobA proteins exist as a MobA-RepB fusion, only the N-terminal 400 aa was considered for comparison with other MobA-related proteins. The protein equivalent to MobC in pTF1 is called MobS. Percentages are percent amino acid sequence identities. GenBank accession numbers are as follows: pMRC01, g3582197; pIP501, L39769; pGO1, g1245474; pSK41, g3676420; pNGR23, P55418; pTi-Sakura, g6498282; pTi-A6NC, g2499023; pTi-C58, g2499022; pTF1, g127224; pIE1107, Z74787; pIE1115, AJ293027; pDN1, Y19120; pIE1130, AJ271879; RSF1010, M28829; pSC101, P14492; pAB6, g4884735.](http://mmbr.asm.org/)

![FIG. 6. Comparison of the regions involved in the mobilization of plasmids pRA2, pTF-FC2, and the IncPα plasmid RP4/RK2. Broken lines linking plasmids show the regions with amino acid sequence similarity.](http://mmbr.asm.org/)
Sizes of Plasmids of the IncQ-Type Family

The single-strand displacement mechanism of replication, together with the plasmid-associated replication proteins, appears to have conferred exceptionally broad-host-range properties to plasmids of this type. In addition, an advantage may be derived from the plasmid being independent of the host priming system for lagging-strand DNA synthesis. A disadvantage of this mechanism of replication is that during replication, up to half of the plasmid will be present as a single-stranded DNA. One may speculate about whether the strand displacement replication mechanism places a limit on the size that may be attained by plasmids that replicate by this process. It is interesting that the natural plasmids which belong to the IncQ family are all fairly small, ranging in size from 5.1 kb (pDN1) to 14.0 kb (pTC-F14). None of these plasmids is more reactive and much less physically stable than double-stranded DNA. One may speculate about whether the strand displacement replication mechanism places a limit on the size that may be attained by plasmids that replicate by this process. It is interesting that the natural plasmids which belong to the IncQ family are all fairly small, ranging in size from 5.1 kb (pDN1) to 14.0 kb (pTC-F14). None of these plasmids are self-transmissible, possibly because the coding capacity required for the synthesis of the conjugation apparatus is too large to be stably accommodated in a replicon of this type. It would be interesting to determine whether there is a difference in the limit to the size of plasmid that can be efficiently supported by a replicon of the strand displacement type compared with mechanisms in which leading- and lagging-DNA-strand synthesis takes place simultaneously.

Relaxase-Primase Fusion

As discussed above, the mobilization regions of the IncQ family plasmid backbone are of two different types (an IncQ-type and an IncP-type); however, in both types the relaxase, which is an essential activity for mobilization, has been fused to a primase, which is required for plasmid replication. A mobilization-replication fusion of this type does not appear to have happened between the IncQ-like mob region and non-IncQ-like replicon of pTF1 or between the IncQ-type mob region and the unique replicon (unrelated to either IncP or IncQ plasmid families) of the non-self-transmissible plasmid pRA2 (45). Why a mob-rep fusion should have occurred in all plasmids of the IncQ family plasmids is unclear. The fusion in plasmid R1162 occurs because the MobA-linked primase is essential for plasmid mobilization (36). However, the mob-rep fusion does not appear to be required for the mobilization of all IncQ-type plasmids. In plasmid pTF-FC2, a ClaI site is naturally located within the coding region of this relaxase-primase fusion.

EVALUATION ASPECTS

Two IncQ-Type Plasmid Groups Identified

Based on a comparison of features such as the amino acid sequence similarities of the RepA, RepB, and RepC proteins, as well as the relatedness of the operon structure, Mob proteins, and oriT areas of the mobilization regions, there are at least two major groups within the IncQ plasmid family. We propose to call these plasmids IncQ groups 1 and 2. If additional groups are discovered, these could be named groups 3, 4, and so on. How many of these major groups may exist is uncertain and possibly awaits the investigation of plasmids in bacteria from environments other than those so far examined. Based on iteron sequence similarity and incompatibility, the two major groups can be subdivided into subgroups termed IncQ-1a for plasmids which have a functional oriV incompatible with RSFI010, IncQ-1b for those with an oriV incompatible with pIE1107, pIE1115, or pDN1; and IncQ-1y for those with an oriV incompatible with pIE1130. We propose that the subgroups of the second group of IncQ-like plasmids should be named IncQ-2a for pTF-FC2 and IncQ-2b for pTC-F14. This scheme is consistent with that currently in use for the IncPα and IncPβ subgroups of IncP plasmids.
fission in such a way that the two activities of this multifunctional protein can be separated. By removal of DNA from either side of the ClaI site, it has been shown that each part can be expressed independently of the other and that only the relaxase portion is required for efficient mobilization of pTF-FC2 and only the primase portion is required for replication (22, 71). This indicates that the two parts of the large fusion protein can function independently of each other. The reason why both parts of the relaxase-primase protein have remained fused in the form of a large open reading frame so as to link the replicon and mobilization properties of the plasmid is still a mystery.

Nucleotide Sequence Composition

The nucleotide composition of the members of the IncQ plasmid family investigated to date is approximately 60% G+C (Table 3). Scholtz et al. (80) showed that the codon bias of the genes comprising the plasmid backbone (replication/maintenance and mobilization functions) was markedly different from that of the genes encoding antibiotic resistance. This was interpreted as indicating that the antibiotic resistance genes had been incorporated more recently in the evolution of RSF1010. The separation of the repC genes from the oriV region by antibiotic resistance genes in RSF1010 but not the other members of the IncQ plasmid family is consistent with the view that the antibiotic resistance genes were inserted after a functional replicon had evolved. In Table 3 we present calculations of the moles percent G+C ratios for the plasmid sequences as a whole as well as separate moles percent G+C ratios for the plasmid backbones and accessory DNA (frequently antibiotic or transposon-like regions). The difference in G+C content for the plasmid backbones compared with the accessory DNA for the IncQ-1α, IncQ-1β, and IncQ-1γ plasmids ranges from 7.7% for RSF1010 to 11.4% for pIE1130. For pTF-FC2, the difference between the accessory DNA (within the 38-bp Tn21-like transposon ends) is only 0.8%. This suggests that insertion of a Tn21-like transposon into pTF-FC2 is an evolutionarily old event or that the source of the transposon was an organism with a G+C ratio close to that of the pTF-FC2 backbone. This second possibility is likely since the G+C content of A. ferrooxidans genome is 59%, which is very close to the average content for pTF-FC2.

TABLE 3. Comparison of G+C contents of whole plasmid, backbones, and accessory DNA of sequenced plasmids of the IncQ family

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Whole plasmid G+C (mol%)</th>
<th>Plasmid backbone G+C (mol%)</th>
<th>Accessory DNA G+C (mol%)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSF1010</td>
<td>60.9</td>
<td>63.9</td>
<td>56.2</td>
<td>7.7</td>
</tr>
<tr>
<td>pIE1107</td>
<td>59.1</td>
<td>62.0</td>
<td>54.1</td>
<td>7.9</td>
</tr>
<tr>
<td>pIE1115</td>
<td>56.2</td>
<td>62.1</td>
<td>51.0</td>
<td>9.1</td>
</tr>
<tr>
<td>pIE1130</td>
<td>57.0</td>
<td>62.2</td>
<td>50.8</td>
<td>11.4</td>
</tr>
<tr>
<td>pDN1</td>
<td>62.3</td>
<td>62.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>pTF-FC2</td>
<td>59.8</td>
<td>60.0</td>
<td>59.2</td>
<td>0.8</td>
</tr>
<tr>
<td>pIE1107</td>
<td>ND</td>
<td>59.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Replicon only.

b NA, not applicable.

c ND, not determined.

Evolution of New Incompatibility Groups

The finding of several IncQ-like plasmids with more than one oriV region and the unidirectional (asymmetrical) incompatibility of these plasmids toward RSF1010 is intriguing. A model for the diversification of plasmid incompatibility has been proposed by Sykora (91), and its relevance to the IncQ plasmid family has been discussed by Tietze (93) and Osborn et al. (59). According to this model, plasmid cointegrate formation can serve as mechanism for the evolution of new incompatibility groups. Cointegrate plasmids with two compatible origins of replication have an advantage over the double-plasmid state, where the two plasmids exist as separate compatible units. Each of the separate plasmids may be eliminated by invasion of an incompatible plasmid, whereas with a cointegrate, the incompatible replicon is saved from elimination by the other replicon. Cointegrate plasmids may allow for the evolution of new rep genes and incompatibility groups because conservative selection of one of the replicons is relaxed. Applying the model to the IncQ plasmid family, the presence of duplicate replicons would allow for the rep genes and the target iterons of one of the replicons to accumulate mutations, resulting in pseudogenes and altered iterons. Should the old rep gene be inactivated or the cointegrate be resolved, the new rep genes and iterons would come under strong selection to function as efficient, independent, and competitive replicon. The result of this process is that an IncQ-like plasmid of a new incompatibility type would be formed.

As pointed out by Tietze (93) plasmids which have two oriV regions, such as pIE1107 and pIE1130, could have been derived from cointegrate formation between two plasmids followed by partial deletion of one of the replicons. An alternate explanation is that the second oriV could have been acquired by recombination with the oriVa-containing region of a different IncQ-type plasmid. Although retention of two oriV regions could be a driving force for further evolution, there is another possible reason for two oriV regions in the case of an environment like piggery manure. This environment would appear to be populated by cells containing a large variety of plasmids, including several types of IncQ or IncQ-related plasmids. A plasmid which retained an oriV with incompatibility to IncQ plasmids but which replicated using an alternate compatible IncQ-like oriV would have a distinct selective advantage in such an environment. Cointegrate plasmids of this type would not be displaced by IncQ plasmids, but since they have an IncQ incompatibility determinant associated with the nonessential oriV, they would be able to reduce the copy number and ultimately displace IncQ plasmids from a host cell. For example, the nonfunctional, RSF1010-identical, oriV-like region of plasmid pIE1107 is able to displace the IncQ plasmid RSF1010 but it is not displaced by RSF1010 because it uses an alternate RSF1010-compatible replicon for its own replication. This could provide a plasmid like pIE1107 (e.g., pIE1115 and pIE1130) with a distinct competitive advantage in a densely populated plasmid-rich environment like piggery manure. It is
interesting that plasmid pDN1, which has a very similar core region to pIE1107, has only a single oriV (Fig. 1), possibly because competition from invading plasmids is not as fierce in the soil (sheep footrot) environment in which it occurs.

There is a tension between the idea of having a second oriV serving as a driving force for evolution or as an additional incompatibility determinant. To serve as an element for evolution, the oriV would need to accumulate mutations, whereas to serve as an additional incompatibility determinant, it would need to remain relatively unaltered so as retain the same incompatibility as the cointegrate plasmids most serious competitors. Interestingly, the secondary oriV regions of pIE1107, pIE1115, and pIE1130 have retained identical iteron sequences and hence the same incompatibility as the IncQ plasmids. Even the interon-flanking region which contains the ssiA and ssiB sites has remained almost free of mutations. It is difficult to know whether this apparent sequence stability is as a consequence of a recent acquisition or whether there is strong selection for an unaltered secondary oriV to enable the plasmid to outcompete plasmids of the same incompatibility group as the extra oriV. Since only the oriV and not a second copy of the rep genes of the second cointegrate has been retained, the additional oriV probably serves as a means of giving a plasmid like pIE1107 a competitive advantage rather than being a means for the evolution of a new replicon.

**Acquisition of Regulation Genes**

Regulation of the genes associated with the replication and mobilization functions of the IncQ plasmids RSF1010 and R1162 has been investigated in detail. Four promoters associated with the expression of these core functions have been identified, as well as an autoregulatory gene termed cac (control of rep AC) (51). Regulation of the other members of the IncQ plasmid family is largely uninvestigated. However, from an examination of the IncQ-like plasmids, only pIE1130 has a gene or open reading frame with sequence similarity to the cac gene or gene product. This implies that the regulatory functions of the others must be substantially different. The 350-bp region, which is located between the rep and mob genes of pIE1107, pIE1115, and pDN1, contains a small 78-aa-protein-encoding open reading frame, which may serve as a rep or mob gene regulator. The G + C content of this region of pIE1107 is 48%, compared with 64% along the mob and rep genes (59). Similarly, it has been pointed out that the G + C content of the gene for the OrfE protein adjacent to the cac gene of RSF1010 differs significantly from the rep and mob genes. This observation has been used to argue that this region has been recently acquired (80). Although evidence of recent acquisition of regulatory genes is not fully convincing, the IncQ plasmid family has more variation in this region than do other regions of their backbone structure.

The IncQ-like plasmid group 2 also possess small genes within the mob-rep intergenic region. Surprisingly, these proteins appear to have nothing to do with replication control but constitute a toxin-antitoxin proteic plasmid stability system named pas (plasmid addiction system) (87). Evidence that the pas genes do not encode regulatory proteins was provided by the introduction of a frameshift mutation within the pasA antitoxin gene. This resulted in very sick E. coli host cells and in a strong selection for the creation of two different types of spontaneous pas deletions. In one of these deletions, almost the whole of the pas system had been lost and the plasmid was able to replicate in E. coli with a copy number that was unaltered from that of the original replicon (87). The source of the pas system is unknown, but at 60%, the G + C content of the pas region is different from that of the rest of the pTF-FC2 plasmid.

In the IncQ plasmid RSF1010, the oriT site is a major site of plasmid regulation including plasmid replication (26). However, this may not apply to all IncQ-type plasmids because although the regulation of mobilization genes of pTF-FC2 also occurs at the oriT region (72), the replicon can function independently of the mobilization region at a copy number that is indistinguishable from that in the intact plasmid.

**Counterselection for Restriction Enzyme Recognition Sites**

DNA which enters a new host during conjugation may be subject to digestion by restriction endonucleases which cleave DNA that has not been modified by cognate methyltransferases (3, 10). Promiscuous broad-host-range plasmids are likely to encounter different restriction modification systems when entering different hosts. One of the strategies that plasmids have acquired to prevent cleavage by restriction enzymes is the use of antirestriction mechanisms (15). However, these tend to target specific restriction enzymes and do not confer universal protection. The elimination of restriction enzyme cleavage sites is a more universal protection mechanism. For example, in the broad-host-range IncP plasmids, type II restriction endonuclease sites are (with two exceptions) particularly underrepresented on the backbone of broad-host-range plasmid RP4 relative to the chromosomes of P. aeruginosa, a natural host bacterium which has a similar G + C ratio (97). It has previously been noted that the replicon of the IncQ-like plasmid pTF-FC2 had remarkably few cleavage sites for the commonly used type II restriction enzymes (21). Rather than examine the sequences of the IncO-like plasmids for restriction enzymes, we have used the strategy of Wilkins et al. (97) and determined the frequency of occurrence of all 64 6-bp sequences with twofold rotational symmetry. These 6-bp palindrome sites are the most common class of recognition sequence for DNA cleavage by type II restriction enzymes. Each plasmid as a whole was searched for the occurrence of these palindromes, and this search was repeated separately for the backbone and accessory DNA regions. The frequency has been calculated as the average number of base pairs per palindrome occurrence and is shown in Table 4. In all four plasmids examined, there is a markedly lower frequency of occurrence of the 64 6-bp palindromes in the plasmid backbone (one site every 137 to 321 bp) compared with the accessory DNA (one site every 57 to 90 bp). This difference in frequency is most noticeable for pTF-FC2. In this plasmid, no 6-bp palindrome occurs more than once, with the exception of TGCCA, which occurs seven times. However, no restriction enzyme has been identified which recognizes this palindrome, and it may be that there has been no selection against its presence. One interpretation of the reduced occurrence of 6-bp palindromes in plasmid backbones is that these structures are relatively ancient compared with the accessory DNA, which in most cases is a
result of recent transposition. Selection for the elimination of restriction enzyme cleavage sites from the backbone structures of plasmids of the IncQ family may have taken place as a consequence of their promiscuous nature, whereas this selection has been absent from the accessory DNA, which has presumably been acquired from narrow-host-range plasmids or chromosomes.

**SUMMARY AND FUTURE DIRECTIONS**

The two major groups of IncQ-like plasmids described in this review were isolated from bacteria growing in two very different environments. It is unlikely that IncQ-like plasmids would be found in bacteria as diverse as the neutrophilic, heterotrophic bacteria associated with medical samples or animal wastes and the acidophilic, obligately autotrophic bacteria associated with inorganic mining environments, if they are not also present in other ecological niches. It may therefore be assumed that other plasmids of the IncQ family will be found in a variety of other environments once these environments are examined for their presence. The Southern hybridization and PCR-based techniques used to screen for IncQ-like plasmids were based on hybridization probes and PCR primers from IncQ-like group 1 plasmids and would almost certainly not be found in bacteria as diverse as the neutrophilic, heterotrophic bacteria associated with inorganic mining environments, if they are not also present in other ecological niches. It may therefore be assumed that other plasmids of the IncQ family will be found in a variety of other environments once these environments are examined for their presence. The Southern hybridization and PCR-based techniques used to screen for IncQ-like plasmids were based on hybridization probes and PCR primers from IncQ-like group 1 plasmids and would almost certainly not have detected IncQ-like group 2 plasmids such as pTF-FC2 or pTC-F14. Likewise, the very successful mating experiments used to capture the IncQ-like plasmids pIE1117, pIE1115, and pIE1130 in *E. coli* were dependent on the plasmids having selectable antibiotic resistance markers and would not have isolated plasmids like pDN1. A comparison of the DNA sequences of all IncQ-like plasmids discovered so far is needed to attempt to identify regions which are sufficiently conserved to be used as hybridization probes or for the design of PCR primers which would detect members of the IncQ plasmids belonging to both known major groups. The most highly conserved regions occur within the *repC* genes, but PCR primers that would detect all the currently known types of IncQ-like plasmids still need to be designed and tested before they can be used to screen for these plasmids in new environments. Alternatively, degenerate primers, possibly used together with an appropriate PCR protocol, are required.

All of the IncQ-like plasmids discovered so far have a backbone consisting of a mobilization region and a plasmid replication maintenance region. Their wide distribution indicates that the replication and mobilization combination characteris-
sistent with this fairly high G+C content (P. aeruginosa, 67%; A. ferrooxidans, 59%; A. caldus, 61 to 63%). However, some of the IncQ-related plasmids were originally isolated from genera with considerably lower G+C contents, such as *Dichelobacter* (45%) or *Salmonella* and *Escherichia* (48 to 52%). In spite of this, the G+C content of the plasmid backbone is uniformly high. Whether this high G+C content has biological significance or is simply a consequence of the evolutionary history of the IncQ-like plasmid family is an unanswered question. Possibly the ancestral IncQ-like plasmid evolved in bacteria with a high G+C content and has been insufficient selection pressure to reverse this. Since the accessory DNA of the IncQ-1 plasmids has a substantially lower G+C content than and codon usage pattern different from the rest of the backbone, these regions must have been acquired from other sources. This suggests that the plasmid must reside for unknown periods in hosts with G+C contents lower than 60 to 64%. It may be that these plasmids are now so promiscuous that the length of time they spend in a host with a low G+C content before again residing in a host with a high G+C content is too short to allow the evolution of a plasmid G+C content that matches that of the host. It will be interesting to monitor this feature in IncQ-like plasmids that may be discovered in the future, especially those isolated from bacteria with G+C contents substantially different from 60 to 64%.

The lack of restriction endonuclease cleavage sites within the plasmid backbone is presumably an adaptation to the ability of the IncQ-like plasmids to spread horizontally through a variety of hosts within a mixed microbial community. However, this lack of restriction endonuclease cleavage sites does not apply to the accessory DNA, and the acquisition of DNA with an abundance of such sites may represent an “Achilles heel” that restricts rapid horizontal plasmid spread. Plasmids like pDN1 which consist only of a plasmid backbone with few restriction endonuclease cleavage sites, may have an advantage in horizontal transfer. It is possible that plasmid pDN1, which does not appear to have accessory DNA and has a very different G+C content from the host in which it was discovered, is a “lightweight” backbone plasmid cruising between hosts. However, the presence of a plasmid with its intrinsic metabolic burden would decrease host cell fitness. Under these conditions, there would be selection pressure for backbone plasmids to acquire accessory DNA that increases host fitness and helps to ensure that plasmid-containing cells are not eliminated from the population. Since the acquisition of accessory genes with their accompanying restriction enzyme cleavage sites would in turn restrict horizontal plasmid transfer, there is likely to be a tension between ease of plasmid transfer and benefit to the host. Natural backbone plasmids like pDN1 and plasmids with accessory genes like RSF1010, pTF-FC2, and others possibly represent nature’s compromise solution to this problem.

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