Polynucleotide Sequence Relationships among Plasmids of the I Compatibility Complex

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SUMMARY

Plasmids determining I pili show a variety of intricate compatibility relationships. None the less, all I plasmids tested showed a 'core' of common DNA sequences of approximately 30×10^6 daltons. Plasmids of the O compatibility group do not appear to specify for the synthesis of I-like pili and yet they held about 20 % of their sequences in common with all tested I plasmids, suggesting a common phylogenetic origin. Members of the I complex (including group O) do not hold a significant proportion of their sequences in common with representative plasmids of other compatibility groups. We propose that the regions of similar polynucleotide sequences of the different I complex plasmids are due to phylogenetic homology between genes governing pili biosynthesis and/or transfer functions. The regions determining compatibility of the I complex plasmids in some cases may be either unrelated to or have diverged from a common ancestor.

INTRODUCTION

Plasmids of the same compatibility group generally have a significant proportion of their polynucleotide sequences in common; plasmids of different groups share few sequences (Guerry & Falkow, 1971; Grindley, Humphreys & Anderson, 1973; see also below). One exception to this general rule has been observed among plasmids of the various compatibility groups whose members determine F-like pili (Hedges & Datta, 1972). These plasmids, belonging to distinct compatibility groups, exhibit extensive genetic complementation (Willets, 1971) and have a significant proportion of their DNA sequences in common (Guerry & Falkow, 1971). In the main, the shared DNA sequences of these plasmids encompass the sequences involved in transfer functions (Sharp, Cohen & Davidson, 1973). Apparently, any set of compatibility groups whose members determine similar pili may be phylogenetically related, at least with respect to the DNA sequences involved in transfer.

Another set of distinct compatibility groups whose members determine a specific type of sex pilus is the I complex (Hedges & Datta, 1973; Datta & Olarte, 1974). Grindley *et al.* (1973) studied four plasmids which determined I pili; three, all mutually incompatible, showed a high level of common DNA sequences, whereas the fourth, which was compatible with the other three, did not hold a significant proportion of its DNA sequences in common with the others.

In this paper, we report the results of DNA–DNA duplex studies between further members of the I complex, as well as polynucleotide sequence relationships among various R-plasmid

compatibility groups. All members of the I complex examined proved to be significantly related.

METHODS

Bacteria. All plasmids to be tested were transferred to the *Escherichia coli* K12 strain J53 to provide a common genetic background.

Media. The media employed were as described by Datta *et al.* (1971).

Transfer of R factors and R-factor compatibility. R-factor transfer methods were those described by Datta *et al.* (1971). Compatibility testing methods were as described by Coetzee, Datta & Hedges (1972) and by Hedges & Datta (1973).

Molecular characterization of bacterial plasmids. Cleared lysates of [³H]thymine (18 Ci/mm, New England Nuclear)-labelled bacteria were prepared as described by Clewell & Helinski (1969). Samples of cleared lysates were diluted 1:1 in TES (0.05 M-NaCl, 0.005 M-EDTA and 0.03 M-tris, pH 8.0) and 100 μ l were immediately layered on top of 5 to 20% neutral or alkaline sucrose gradients. Neutral sucrose gradients contained 0.01 M-potassium phosphate, 0.5 M-NaCl, pH 7.0, and were generated over a 0.1 ml shelf of 60% sucrose in 1.27 $\times$ 5.08 cm cellulose nitrate tubes. ¹⁴C- λ -phage DNA monomers (34 s) were added as a sedimentation marker in neutral sucrose gradients and the tubes were centrifuged in the SW50.1 rotor of a Spinco L2-50 preparative ultracentrifuge for 30 min at 149 000 g at 20 °C. Five to 20% alkaline sucrose gradients contained 0.5 M-NaCl, 0.02 M-EDTA and 0.3 M-NaOH, pH 12.5. Alkaline sucrose gradients were centrifuged in the SW50.1 rotor for 20 min at 117 000 g at 25 °C. The fractionation of gradients and radioisotope counting were carried out as described previously (Silver & Falkow, 1970). The molecular weight of open circular and covalently closed circular plasmid DNA in neutral sucrose gradients was calculated relative to the ¹⁴C- λ marker as described previously (Silver & Falkow, 1970). The molecular weight in daltons, *M*, of covalently closed plasmid DNA observed in alkaline sucrose gradients was calculated from the relationship $s_{alk} = 0.19M^{0.397}$ derived in Seattle, where s_{alk} is the sedimentation coefficient in alkaline gradients. The molecular weight of a given plasmid DNA determined by sedimentation in neutral and alkaline sucrose agreed in all cases within $\pm 2 \times 10^6$ daltons. Each plasmid was assayed by these procedures at least three times.

Plasmid DNA was further purified for the determination of guanine + cytosine (G + C) content, electron microscopy and for DNA-DNA duplex studies (see below) by mixing [³H]thymine-labelled cleared lysates with a concentrated solution of CsCl (American Potash Co.) in TES and ethidium bromide (Calbiochem; final concentration 250 μ g/ml). The density was adjusted to 1.625 g/cm³ (refractive index 1.3925). The mixture was centrifuged at 25 °C for 40 to 60 h at 129 000 g in the fixed angle type 65 rotor of an L3-50 preparative ultracentrifuge and fractionated as described by Guerry, van Embden & Falkow (1974*b*). Material from the dense covalently closed circular DNA peaks was pooled, the ethidium bromide removed by extraction with an equal volume of isopropanol and the DNA was dialysed against TES or other suitable buffer solution. A sample of pooled plasmid peaks was re-examined on alkaline and neutral sucrose gradients as described above.

The G + C content of plasmid DNA was determined by adding about 3000 c.p.m. of purified ³H-labelled plasmid DNA to a concentrated solution of CsCl in TES at a density of 1.710 g/cm³ (refractive index 1.4010) together with ¹⁴C- λ DNA (49% G + C) or ¹⁴C-NSF1010 plasmid DNA (55% G + C; Guerry *et al.* 1974*b*). The mixture was centrifuged for 40 to 60 h at 129 000 g in a fixed angle type 65 rotor and fractionated as described previously (Guerry *et al.* 1974*b*). The density of the banded ³H plasmid DNA was calculated from the linear relationship between refractive index and density from which the mole fraction of G + C of the plasmid was estimated (Silver & Falkow, 1970).

The electron microscopy of the isolated plasmid DNA and the measurement of contour length of open circular molecules was performed as described by Guerry *et al.* (1974*b*).

The measurement of DNA-DNA duplexes. The determination of the degree of polynucleotide sequence relationship in common between plasmids was assayed by both a hydroxyapatite (HA) method (Brenner *et al.* 1969; Guerry & Falkow, 1971) and by the use of the single-strand specific S1 nuclease of *Aspergillus oryzae* (Crosa, Brenner & Falkow, 1973). As described previously (Crosa *et al.* 1973) both methods give identical results for the estimation of plasmid DNA homo- and heteroduplexes. Purified [³H]thymine-labelled plasmid DNA was sheared to a molecular weight of 250 000 daltons (Brenner *et al.* 1969) and denatured by heating at 100 °C for 5 min. Most labelled plasmid preparations possessed a specific activity of approximately 4×10^5 c.p.m./μg. Unlabelled DNA from the *E. coli* J53 F⁻ strain and *E. coli* J53 containing appropriate plasmids was extracted as described by Guerry & Falkow (1971). These DNA preparations were employed, after shearing and denaturation, as a source of plasmid DNA in the duplex studies. The actual amount of plasmid DNA in such unlabelled, unfractionated host material was, of course, not known precisely but was estimated to be at least 2 to 4 % of the total DNA in all cases (Guerry & Falkow, 1971).

DNA-DNA reassociation reactions assayed by the HA method were performed by incubating approximately 0.1 μg of sheared, denatured, purified plasmid DNA (3000 c.p.m.) with 400 μg of the unfractionated, sheared, denatured bacterial preparations in 1 ml of 0.14 M-phosphate buffer pH 6.8. These DNA mixtures were incubated at 75 °C and 60 °C for long enough to achieve essentially complete reassociation in the homologous reaction. The incubation time employed for each plasmid varied to some extent depending upon the molecular weight (i.e. complexity) of the plasmid DNA. We were generally guided in the selection of the time of incubation by calculating the $c_0t_{\frac{1}{2}}$ (Brenner & Falkow, 1971; Brenner *et al.* 1969; Guerry & Falkow, 1971) of the DNA according to the empirical relationship:

$$\text{Molecular weight, } M (\text{daltons}) = c_0t_{\frac{1}{2}} \times 377 \times 10^6$$

derived in this laboratory (Haapala & S. Falkow, unpublished observations), where c_0 is the initial concentration of single-stranded DNA and $t_{\frac{1}{2}}$ the half-time of the reaction. Each mixture was incubated for an equivalent of $20c_0t_{\frac{1}{2}}$. The fractionation of unreacted single-stranded DNA from reassociated DNA duplexes was as described previously (Brenner *et al.* 1969; Guerry & Falkow, 1971). Under our experimental conditions labelled DNA strands incubated alone had reassociated less than 2 %, while more than 85 % of the labelled DNA had reassociated with its homologous unlabelled DNA preparation. The reassociation of labelled plasmid DNA with the *E. coli* F⁻ strain (chromosome control) was included in each experiment and varied from 2.9 to 13 % depending upon the plasmid. This value was subtracted from all reactions. The degree of duplex formation of labelled DNA with its homologous source corrected for the F⁻ contribution was taken as 100 % and all heterologous reactions, similarly corrected, were calculated relative to the homologous reaction.

The assay of DNA-DNA duplexes by the S1 endonuclease method was performed by incubating about 0.1 μg of purified, sheared, denatured plasmid DNA (2000 to 3000 c.p.m.) with 150 μg of sheared, denatured bacterial DNA in 1 ml of 0.42 M-NaCl. Reassociation was carried out at 75 °C for a suitable time and stopped by icing. Samples (0.2 ml) of the reassociation mixture were removed into duplicate tubes containing (in a total volume of 1 ml): 0.1 mM-ZnSO₄, 0.15 M-NaCl, 30 mM-sodium acetate buffer pH 4.5, and 20 μg sheared denatured calf thymus DNA. S1 endonuclease (95 units in 10 μl) was added to one of the

tubes and buffer added to the other. Mixtures were incubated at 50 °C for 20 min and the reaction was terminated in an ice bath followed by the immediate addition of 0.25 ml ice-cold trichloroacetic acid. The trichloroacetic acid precipitate was collected on membrane filters, dried at 70 °C, and the radioactivity determined in a liquid scintillation spectrometer. The homologous reaction was estimated by determining the percentage of trichloroacetic acid-insoluble counts remaining after incubation with the S1 endonuclease as compared with the nuclease-free control tube. For each labelled preparation it was also necessary to determine what fraction of denatured plasmid DNA was resistant to the S1 endonuclease and whether any double-stranded endonucleolytic activity was present (Crosa *et al.* 1973). As above, an F⁻ chromosome control was included in each experiment and taken as 0% duplex formation, whereas the corrected homologous reaction was taken as 100%. The uncorrected degree of duplex formation for the homologous reaction by the S1 endonuclease assay was generally 88 to 90%, whereas the uncorrected degree of duplex formation with the chromosomal control was between 8 and 13%.

RESULTS

Polynucleotide sequence relationships among plasmids unrelated to the I complex

Table 1 is a summary of the molecular properties and DNA relatedness of selected representatives of the FI, FII, N, W, P, X and the prototype I (I α) compatibility groups as well as for the non-transmissible R plasmid, NSF1010, of unknown compatibility type, which exists as a multi-copy pool within *E. coli*. Even this limited selection demonstrates the wide range of molecular size and mole fraction G + C which one observes among plasmids. For example, transmissible R plasmids of the W group were found to be as small as 20×10^6 daltons as contrasted to the better known FII and I α plasmids which are in the 50×10^6 to 70×10^6 dalton range. Moreover, the mole fraction G + C of individual plasmids range from 0.45 to 0.62. None the less, as noted by others (Guerry & Falkow, 1971; Grindley *et al.* 1973), it may be seen that the plasmids of any particular compatibility class were found to be relatively homogeneous with respect to their general molecular size, G + C content, and common polynucleotide sequences. Plasmids of different compatibility classes showed less than 15% duplex formation with each other except for the FI and FII classes noted earlier (Guerry & Falkow, 1971; Sharp *et al.* 1973). The reassociation experiments were all carried out at 75 °C, a temperature which sets stringent criteria for the formation of DNA duplexes and discriminates against imperfectly matched base pair sequences (Brenner & Falkow, 1971). The identical reactions carried out at 60 °C, a temperature which permits the identification of sequences containing up to 17% imperfectly matched base pairs (Brenner & Falkow, 1971), gave virtually identical results. Thus, the degree of nucleotide sequence similarity (even when the degree of reassociation was minimal) appeared to involve well-matched nucleotide sequences.

The molecular nature and compatibility relationships among I-complex plasmids

Plasmids which determine I pili have been subdivided by their compatibility properties (Hedges & Datta, 1973). The compatibility group represented by the original I plasmids, R144, R64 and ColIb-P9 was termed I α . Subsequently, other R factors determining I pili were described which were compatible with I α plasmids or with each other. Thus, R483 (the prototype of group I β) showed mutual incompatibility with I α in certain crosses, but stable doubles have been observed. Another I plasmid, JR66a (the prototype of group I ω) was incompatible with plasmids of both I α and I β groups. Yet another plasmid, R621a

Table 1. Polynucleotide sequence relationships between plasmids of various compatibility groups

Unlabelled R-factor DNA	Com- patibility groups	Resistance markers*	10 ⁻⁶ × molecular weight† (daltons)	Mole fraction G + C‡	No. of plasmid copies per cells	Extent of DNA-DNA duplex formation with [³ H]thymine R-factor DNA						Reference¶
						R144	R144d19	N3	RP4	Sa	NSF1010	
R144	Iα	Tc Km	63	0.50	1-3	100	8	5	<1	2	7	Hedges & Datta (1973)
R64	Iα	Tc Sm	72	0.50	1-3	78	—	—	—	—	—	Hedges & Datta (1973)
R144d19	FII	Ap Sm Cm Km Su	65	0.515	1-3	6	100	6	9	14	9	Meynell & Datta (1967)
F	FI	—	63	0.49	1-3	—	43	—	—	—	—	Datta & Hedges (1971)
N3	N	Sm Tc Su	32	0.49	1-3	2	8	100	1	10	10	Datta & Hedges (1971)
R15	N	Sm Su	40	0.49	1-3	—	—	75	—	—	—	Datta & Hedges (1971)
Sa	W	Sm Cm Km Su	25	0.62	3-5	5	4	8	4	100	<1	Datta & Hedges (1972)
R388	W	Su Tp	21	0.62	3-5	—	—	—	—	79	—	Datta & Hedges (1972)
R7K	W	Ap Sm	20	0.62	3-5	—	—	—	—	75	—	Coetzee <i>et al.</i> (1972)
R6K	X	Ap Sm	25	0.45	15-20	1	8	1	2	1	<1	Hedges, Datta, Coetzee & Denni- son, 1973
RP4	P	Ap Tc Km	34	0.58	1-3	3	—	4	100	3	<1	Datta <i>et al.</i> (1971)
NSF1010	?	Su Sm	5	0.55	10-20	—	—	—	—	—	100	Guerry <i>et al.</i> (1974)

* Abbreviations: Ap, ampicillin; Sm, streptomycin; Tc, tetracycline; Cm, chloramphenicol; Km, kanamycin; Su, sulphonamide; Tp, trimethoprim.

† The molecular weight of each plasmid species was determined either by sedimentation in sucrose gradients or by the examination of circular DNA in the electron microscope.

‡ The mole fraction of G + C was determined in preparative neutral CsCl gradients of [³H]thymine DNA purified by dye buoyant density equilibrium centrifugation. The G + C content was calculated relative to [¹⁴C]thymine-labelled *E. coli* DNA which was taken as 1.710 g/cm³, 0.50 mole fraction of G + C.

§ The number of plasmid copies per cell was calculated from the proportion of covalently closed circular DNA molecule present in whole-cell sarkosyl lysates centrifuged to equilibrium in CsCl-ethidium bromide. The *E. coli* chromosome was assumed to be 2.5 × 10⁶ daltons for these calculations.

|| The degree of DNA-DNA duplex formation was assayed on hydroxyapatite and by the S1 endonuclease method as described in Methods. In every case, the degree of duplex formation was corrected for the reaction obtained with *E. coli* F⁻ DNA and the results were calculated relative to the homologous reaction. The actual extent of binding of ³H-R144, ³H-R144d19, ³H-RP4, ³H-Sa and ³H-NSF1010 DNA with their homologous unlabelled DNA was 92, 50, 87, 89, 86 and 89 %, with an observed F⁻ contribution of 7.9, 13.2, 5.4, 2.3, 3.2 and 2.2 %, respectively.

¶ The references refer to the classification of each plasmid in a given compatibility group.

Table 2. *Compatibility relationships among members of the I complex*

Plasmid in donor cell	Plasmid in recipient cell					
	I α R144	I β R483	I γ R621a	I ω JR66a	I ζ R805a	O R724
R144	—	+	+	—	—	+
R483	—	—	+	—	—	+
R621a	+	+	—	+	+	+
JR66a	—	—	+	—	—	+
R805a	—	—	+	—	—	—
Collb-P ₉	—	+	+	—	—	+
R724	+	+	+	+	—	—
R ¹⁶	+	+	+	+	—	—

+, Compatible; —, incompatible.

(representing group I γ) was found to be compatible with all of the other naturally occurring plasmids of the I complex.

The R factor R805a (Datta & Olarte, 1974) determined I pili and was incompatible with all other I pilus-determining plasmids tested save R621a. Moreover, R805a was incompatible with plasmids of compatibility group O which do not determine pili capable of acting as receptors for the I pilus-specific phage If₁ (Datta & Olarte, 1974). The compatibility relationships of plasmids of the I complex are summarized in Table 2, while the molecular properties of the I complex and O group plasmids which have been examined are listed in Table 3. Two further groups of I pilus-determining plasmids, I δ and I ϵ , have been designated (Jobanputra & Datta, 1974; Hedges, Datta, Kontomichalou & Smith, 1974), but when plasmid DNA representing the prototypes of these groups was examined each was found to contain at least two molecular species. Since we do not yet know whether the drug resistance genes are carried on the I pilus-determining molecular species, we defer further consideration of these groups. Also included in Table 3 are plasmids Δ , TP102, TP110 and TP114 described by Grindley *et al.* (1973).

Polynucleotide sequence relationships among plasmids of the I complex

Table 4 shows the results of DNA-DNA duplex studies among plasmids of the I complex as well as two plasmids included in compatibility group O. Within the I complex there was a significant proportion of shared polynucleotide sequences between every pair of plasmids tested. There was, however, no correlation between the degree of duplex formation and the compatibility patterns. An extreme example was R805a, which was incompatible with group O and with all I pilus-determining plasmids except I γ . Yet, of all plasmids tested, R621a (I γ) showed the highest number of shared polynucleotide sequences with R805a, whereas R805a showed less sequences in common with group O than did several other members of the I complex. The two O group plasmids tested, R¹⁶ and R724, showed greater than 90 % DNA relatedness and also shared from 28 to 40 % of their sequences with all plasmids of the I complex that were examined. Other studies (not shown here) revealed that labelled R724 fragments failed to reassociate to a significant degree (< 10 %) with plasmids of the FI, FII, N, W, P or X compatibility groups. [It should be noted that Grindley *et al.* (1973) observed significant DNA relatedness between three of four I pilus-determining plasmids and R plasmids of group B. Datta & Olarte (1974) have outlined epidemiological evidence for supposing that the plasmids we designate as group O may be the same as group B.]

Table 3. *Plasmids of the I complex*

Compatibility group	Plasmids	Markers*	Ifi increase†	10 ⁻⁶ × molecular weight* (daltons)	Mole fraction G+C*	References‡
I α	R144	Tc Km	+	62	0.50	Hedges & Datta (1973)
I α	ColIb-P9	ColIb	+	65	0.50	Hedges & Datta (1973)
I β	R483	Sm Tp	+	55	—	Hedges & Datta (1973)
I γ	R621a	Tc	+	65	0.51	Hedges & Datta (1973)
I ϵ	R721	Sm Tp	+	46, 30	—	Jobanputra & Datta (1974)
I δ	RM413	Ap	+	50, 30	—	Hedges <i>et al.</i> (1974)
I ζ	R805a	Km	+	48	—	Datta & Olarte (1974)
I ω	JR66a	Sm Km	+	57	0.50	Hedges & Datta (1973)
O	R ¹⁶	Ap Sm Tc Su	—	69	0.50	Datta & Olarte (1974)
O	R724	Cm Sm Tc Su	—	58	—	Datta & Olarte (1974)
I ₁	Δ §	—	+	59	—	Grindley <i>et al.</i> (1973)
I ₁	TP102§	Km	+	59	—	Grindley <i>et al.</i> (1973)
I ₁	TP110§	Km	+	65	—	Grindley <i>et al.</i> (1973)
I ₂	TP114§	Km	+	41	—	Grindley <i>et al.</i> (1973)

* See footnotes to Table 1.

† The propagation of Ifi on plasmid-bearing strains was determined as described by Hedges & Datta (1973).

‡ The references refer to the initial observations placing these plasmids in the I compatibility group.

§ Data reproduced from Grindley *et al.* (1973) and included for comparison; the relationship of the I₁ I₂ classification to ours has not been tested.

Table 4. *Polynucleotide sequence relationships among plasmids of the I complex*
DNA-DNA duplex formation with [³H]thymine labelled:

Unlabelled plasmid DNA	I α (R144)	I β (R483)	I ω (JR66a)	I ζ (R805a)	O (R724)
I α R144	100	73.2 $\pm$ 2.3 (39.8 $\times 10^6$)	70.0 $\pm$ 4.0 (39.9 $\times 10^6$)	71.1 $\pm$ 2.9 (35.3 $\times 10^6$)	28 $\pm$ 2.7 (16.1 $\times 10^6$)
I α Coll	(62 $\times 10^6$)	67.4 $\pm$ 1.2 (37.1 $\times 10^6$)	—	—	—
I β R483	81.4 $\pm$ 2.7 (50.5 $\times 10^6$)	100	60.0 $\pm$ 4.4 (34.2 $\times 10^6$)	79.3 $\pm$ 2.1 (37.8 $\times 10^6$)	35 $\pm$ 3.2 (20.1 $\times 10^6$)
I γ R621	65.6 $\pm$ 3.8 (40.7 $\times 10^6$)	(55 $\times 10^6$)	55.5 $\pm$ 3.3 (31.6 $\times 10^6$)	84.4 $\pm$ 1.4 (40.3 $\times 10^6$)	39.5 $\pm$ 3.2 (22.7 $\times 10^6$)
I ω JR66a	64.5 $\pm$ 3.4 (40.7 $\times 10^6$)	66.8 $\pm$ 5.2 (36.7 $\times 10^6$)	100	67.1 $\pm$ 2.2 (32.0 $\times 10^6$)	40 $\pm$ 4.2 (23.0 $\times 10^6$)
I ζ 805a	72.9 $\pm$ 2.9 (45.2 $\times 10^6$)	57.0 $\pm$ 4.2 (31.4 $\times 10^6$)	64.5 $\pm$ 4.1 (36.8 $\times 10^6$)	100	28 $\pm$ 2.4 (16.1 $\times 10^6$)
O R724	58.2 $\pm$ 2.9 (36.1 $\times 10^6$)	—	35.0 $\pm$ 3.2 (19.9 $\times 10^6$)	22.1 $\pm$ 1.1 (10.5 $\times 10^6$)	100
O R16	19.7 $\pm$ 2.3 (12.2 $\times 10^6$)	—	24.5 $\pm$ 3.2 (13.9 $\times 10^6$)	18.9 $\pm$ 0.9 (9 $\times 10^6$)	96 $\pm$ 3.6 (52.9 $\times 10^6$)
	16.0 $\pm$ 1.3 (9.9 $\times 10^6$)	—			

The degree of DNA-DNA duplex formation was assayed by the S1 endonuclease method as described in Methods. In every case the degree of duplex formation was corrected for the reaction obtained with *E. coli* F- DNA and the results were calculated relative to the homologous reaction. The actual extent of binding of ³H-R144, ³H-R483, ³H-JR66a, ³H-805a and ³H-R724 DNA was 92, 90, 93, 92.6 and 89 % with an F- contribution of 7.9, 7.3, 8.4, 10.1 and 6.4 %, respectively. The results are the averages of at least four separate determinations done in duplicate. The molecular equivalents for each reaction are shown in parentheses, and were calculated as if the proportion of the total plasmid DNA forming DNA-DNA duplexes were a single continuous length of DNA.

DISCUSSION

Plasmids determining F-like pili (identified by their ability to act as receptors for F-specific phages) constitute at least five compatibility groups (Hedges & Datta, 1972; Datta, unpublished). Those determining I pili (identified by their ability to act as receptors for phage If1) show a variety of compatibility patterns (Table 2). We refer to the former plasmids as constituting the F complex and the latter as the I complex. (We include plasmids of group O in the I complex although they do not determine I pili; see below.) The compatibility patterns of the two complexes are dissimilar. Every F pilus-producing plasmid so far tested falls unambiguously into one or other of the five compatibility groups of the F complex, although these are, to some extent, complicated by 'hierarchies' whereby two plasmids of a single group are asymmetrically eliminated from doubles (MacFarren & Clowes, 1967; Datta, Hedges, Becker & Davies, 1974). Most of the plasmids of the F complex belong to groups FI and FII; each of the other three groups has only a single known member. Thus, almost all the studies on F-complex plasmids have focused upon members of the former groups.

The compatibility relationships within the I complex are more intricate (Table 2). Not only are there cases where a single plasmid (e.g. R805a) is incompatible with both of a pair of plasmids, themselves mutually compatible (e.g. R144 and R724), but within a single group, pairs of plasmids show very different patterns of co-existence. In some cases elimination is efficient and rapid, in others prolonged ('metastable') co-existence of pairs of incompatible plasmids is observed (Guerry, Falkow & Datta, 1974a).

The major exceptions to the general rule that plasmids of different compatibility groups have largely unrelated polynucleotide sequences are found among plasmids of the F and I complexes. Among plasmids of the compatible groups FI and FII, it has been definitively shown by electron microscope heteroduplex studies (Sharp *et al.* 1973) that sequences shared in common are restricted to contiguous stretches of DNA involved in pilus biosynthesis and transfer function (about 37 % of the sequences of F). If one performs similar DNA-DNA duplex studies between a transfer-deficient F factor, carrying a deletion removing all genes known to be required for transferability, and an FII plasmid, the degree of duplex formation falls to less than 15 % sequence similarity which is not very different from that usually observed between members of different compatibility classes (Falkow, unpublished observations). These data, of course, imply that the genes governing plasmid compatibility lie outside the *tra* region of the FI and FII plasmids; recent genetic observations have confirmed this implication and place the incompatibility genes adjacent to those thought to be involved in DNA replication (N. S. Willetts, personal communications). This finding is consistent with the view that incompatibility involves competition for specific maintenance sites which govern replication and the distribution of daughter replicons (Jacob, Brenner & Cuzin, 1963). Recent studies (LeBlanc & Falkow, 1973; Hershfield, LeBlanc & Falkow, 1973) also indicate that incompatibility may be reflected in the inhibition of replication of one of the plasmid pairs at a membrane site.

The simplest interpretation of the DNA-DNA duplex findings within the I complex is to assume that all the plasmids tested share a 'core' of common DNA sequences of approximately 30×10^6 daltons. Presumably, the major fraction of these shared sequences represents common genes determining the synthesis and regulation of I pili. The only exception is the R plasmid TP114, which, although producing I pili, has no significant DNA sequences in common with other plasmids of the I complex (Grindley, Grindley & Anderson, 1972; Grindley *et al.* 1973). This result stands in sharp contrast to our findings and may imply

that there are at least two genetically distinct pathways for the determination of receptors for the I-specific phages.

On this interpretation, the patterns of shared polynucleotide sequence within the I and F complexes are similar. We propose that the regions of similar polynucleotide sequences of the different I (or F) complex plasmids indicate phylogenetic homology between the transfer genes. The incompatibility regions of the plasmids of a complex may be either entirely unrelated or have diverged very extensively from a common precursor. The evolutionary pattern of the lambdoid phages is perhaps analogous. A pair of lambdoid phages (e.g. λ and 434) may show great overall similarity but the immunity regions (the major regulatory genes controlling directly or indirectly the activity of all the other genes of the phage) are almost completely unrelated by the polynucleotide duplex test (Westmoreland, Szybalski & Ris, 1969).

The O group of plasmids do not specify I-like pili by our criterion of identification, which is the ability to adsorb and propagate the filamentous DNA phage If₁ (Meynell & Lawn, 1968). Yet, the O group plasmids tested share about 20% of their sequences (roughly 10×10^6 daltons) in common with all tested members of the I complex and are incompatible with R805a (I ζ). Datta & Olarte (1974) proposed that R805a was a hybrid plasmid containing separate genes for the compatibility properties of I ω and O in a single replicon because there was no evidence, other than incompatibility with R805a, of a relationship between O plasmids and the I complex. Our finding that all I-complex plasmids tested are related in some degree to the O group suggests that group O and I plasmids have a common phylogenetic origin. Certainly, the failure of O plasmids to support the growth of phage If₁ need not rule out the presence of I pili, since there is ample evidence with the F-like plasmids that closely related pili may differ significantly in serological properties (Lawn & Meynell, 1970) or by differential adsorption of isometric RNA phages and filamentous DNA phages (Meynell, Meynell & Datta, 1968; Dennison & Hedges, 1972). Hence, the pili specified by O group plasmids may be I-like in their transfer functions despite their inability to adsorb the one available class of I pilus-specific phage. However, further genetic and molecular analyses are required to define the nature of the DNA sequences shared by I and O plasmids.

Although we propose that the region of shared polynucleotide sequences among plasmids of the I complex (as among those of the F complex) is limited to the region of the transfer genes, these plasmids do show a number of general similarities (Tables 1 and 3). These are emphasized by a comparison with plasmids of group W (Sa, R388 and R7K). First, the molecular weights of I complex plasmids are 40×10^6 to 70×10^6 daltons whereas those of group W are 20×10^6 to 25×10^6 daltons. Then, although we believe that the compatibility regions of, say, I and O plasmids are dissimilar, the systems regulating the replication of plasmids of the I complex are functionally similar in that all ensure a ratio of 1 to 3 copies of the plasmid per chromosome, whereas W plasmids show a significantly higher ratio. Finally, the % G+C of the DNA is characteristic; all I complex plasmids tested have a G+C content close to 50% (including R¹⁶, a member of group O) whereas all the plasmids of group W have G+C contents of 62%.

Thus, the plasmids constituting the I complex have overall similarities in addition to the sharing of transfer genes.

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