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Polynucleotide Sequence Relationships Among Some Bacterial Plasmids

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The deoxyribonucleic acid of F-like plasmids appear to share a high degree of nucleotide similarity with each other but are not highly related to I-like plasmids.

Recent studies divide transmissible plasmids into two types, F-like and I-like, referring to whether they determine the synthesis of sex pili resembling those produced by cells harboring the classical F-factor, or those produced by cells carrying the Col I factor (4, 5). This distinction has been sufficient to unequivocally classify approximately 85% of transmissible plasmids studied thus far into one or the other of these two major classes. Yet, the identification of a large variety of characteristics carried by transmissible plasmids suggests that they have likely had different genetic histories. We studied the polynucleotide sequence relationships of several plasmids of the same class to determine to what extent they share a basic similarity in genetic organization.

A single F-like R-factor, R1*drd*, whose molecular properties were recently characterized (6) in our laboratory was employed as a source of radio-labeled deoxyribonucleic acid (DNA). R1*drd* DNA labeled with ^{3}H-thymine was prepared by two methods: (i) from mating mixtures (6) and (ii) fractionation of satellite DNA from intergeneric hybrids (3). Each plasmid to be studied was transferred to a strain of *Escherichia coli* K-12 F$^-$, W1485, to provide a common genetic background. The DNA was extracted from these strains and employed, unfractionated, as a source of plasmid DNA in DNA-binding experiments. The actual amount of plasmid DNA in the unlabeled host material was estimated to be from 2 to 4% of the total DNA on the basis of the amount of covalently closed circular material which was present. The detection of nucleotide sequence relationships between the labeled R1*drd* DNA and the unlabeled plasmid DNA was determined on hydroxyapatite by a method described in detail previously (1). Both labeled and unlabeled DNA preparations were sheared by sonic treatment to an average molecular weight of $2.5 \pm 0.3 \times 10^5$ daltons and denatured by heat. Ordinarily, 0.002 μg of ^{3}H-thymine-labeled R1*drd* DNA (2×10^5 counts per min per μg) per ml was incubated with 300 μg of the unfractionated DNA preparations per ml. This was equivalent to 0.002 μg of R1 DNA per ml incubated with from 6 to 11 μg of unlabeled plasmid DNA per ml and, hence, there was a large excess of unlabeled sequences present to hybridize with the labeled plasmid DNA. Incubations were performed in 0.14 M sodium phosphate buffer (*p*H 6.8) at both 60 and 73 C for a time (usually 16 hr) sufficient to achieve essentially complete reassociation. Reassociated double-stranded duplexes were discriminated from unassociated single-stranded DNA on hydroxyapatite by elution with different buffer concentrations, and the proportion of reassociated and unbound label was determined by counting in a liquid scintillation counter.

Table 1 summarizes the genetic and molecular properties of the plasmids studied and the degrees of nucleotide sequence similarity which were measured at 75 C relative to R1*drd* DNA. The indicated thermal binding index (TBI), which is the ratio of the relative reassociation at 60 C to the relative reassociation at 75 C, served as a rough measure of the presence or absence of highly related material in reassociation experiments (1). The R1*drd* DNA was reassociated with the DNA extracted from *E. coli* K-12 F$^-$ to determine the degree of nucleotide similarity between this R factor, and the host chromosome. There was a slight, but nonetheless significant, degree of nucleotide similarity between R factor and the *E. coli* chromosome. The low TBI indicated that this relationship was comprised predominantly of imprecise sequences; yet, on a quantitative basis, the reactions between R1 and the other plasmids shown in Table 1 are likely to be slightly low in some cases, because of competition for labeled sequences held in common by both the host chromosome and the unlabeled plasmid. A derivative of R1*drd*, termed RTF, was also included (7). This derivative has 23%

TABLE 1. *Polynucleotide sequence relationships among bacterial plasmids*

Source of DNA	F-like[a]	I-like[b]	Mol wt[c] (daltons)	Relative binding at 75 C (%)	TBI[d]
R1*drd* (R-*sul,str,cam,kan,amp*)[e]	+	−	65	100	1.00
RTF (R)	+	−	50	73.5 ± 2.7	1.05
222 (R-*sul,str,tet,cam*)	+	−	67	73.5 ± .2	.92
F	+	−	70	37.5 ± 1.7	4.96
Ent-3	+	−	?	55.6 ± 1.6	
N-3 (R-*sul,str,tet*)	−	+	46	14.7 ± 4.0	.76
R-144 (R-*kan*)	−	+	42	16.4 ± 1.8	.90
Col I_{b9}	−	+	63	17.9 ± 1.6	.89
E. coli K-12 F^-	−	−	2,500	4.3 ± 2.7	.29

[a] Transfer agent determines sex pili with phage adsorption characteristic of the classical F agent.

[b] Transfer agent determines sex pili with male phage adsorption characteristic of the Col I_{b9} agent.

[c] Molecular weights were estimated from the sedimentation of supercoiled plasmid DNA in alkaline sucrose gradients (6). Figures shown to be multiplied times 10^6.

[d] Thermal binding index = relative binding at 75 C/relative binding at 60 C.

[e] R refers to the transfer agent of the R factor; *sul*, *str*, *tet*, *cam*, *kan*, and *amp* refer to resistance to sulfonamide, streptomycin, tetracycline, chloramphenicol, kanamycin, and ampicillin, respectively.

less DNA than R1*drd* and has lost all traces of drug-resistance determinants while, nonetheless, retaining full infectivity and sensitivity to male phages (7). The relative degree of binding of R1*drd* DNA to the *E. coli* RTF DNA preparation was found to be 73.5 ± 2.7%, which is in reasonable agreement with the theoretical expectation. The R1*drd* and the classical sex factor, F, were found to share 37.5 ± 1.6% nucleotide sequence relationships which were precise by the criterion of the TBI. The degree of similarity presumably reflects the similarities of their sex factor activity. Recent electron microscopy studies by Cohen et al. (2) on purified plasmid-plasmid duplexes gave a value of about 42% between F and R1. These quantitatively similar findings further provided information that the sequences in common between F and R1 were contiguous. Two other F-like plasmids, the R-factor 222 and an enterotoxin plasmid (*ent*), also showed a high degree of precise nucleotide sequence relationships with R1*drd* DNA. The *ent* plasmid has not been characterized fully at either the molecular or genetic level but is F-like by the criterion of male phage susceptibility and F inhibition. Preliminary studies also indicate that virtually all F-like R factors share a similar high degree of sequence complementarity, no matter what their origin of isolation. In marked contrast, the Col I factor and, in addition, two I-like R factors studied possessed only a limited degree of nucleotide sequence similarity with R1*drd* DNA. Both I-like R factors studied, N3 and R-144, share resistance to one or more drug-resistance determinants carried by R1*drd*. The duplexes formed between these I-like plasmids and R1*drd* DNA showed a higher thermal stability than that observed for homologous R1*drd* duplexes. This implied that the sequences in common were higher in guanine plus cytosine (GC) than the overall composition of R1 itself. Previous studies indicate that the drug-resistance genes possess, on the average, a higher GC composition than the transfer fraction of an R factor (3, 7). The increased thermal stability, therefore, may suggest that most of the sequence similarity between the I-like R factors and R1*drd* are in regions specifying drug-resistance genes. Thus, it may be that, whereas the sex factor genes of I-like and F-like R factors are quite different, some of the drug-resistance determinants may be derived from the same source. In contrast, the I-like element, Col I, forms duplexes with R1*drd* DNA which showed slightly less thermal stability than the homologous R1*drd* reaction.

These preliminary studies indicate that the distinction between F-like and I-like plasmids is clearly reflected not only in the type of sex pilus but also with respect to overall genetic fine structure. The data further suggest a common ancestral origin for the F-like plasmids which were studied. Presumably this may apply to all F-like plasmids.

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