The Molecular Relatedness of R Factors in Enterobacteria of Human and Animal Origin

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SUMMARY

The molecular length and DNA homology of R factors isolated from enterobacteria of human and animal origin have been examined. DNA from plasmids of the same compatibility group, whether of human or animal origin, is indistinguishable, after allowance has been made for the regions coding for different antibiotic resistances. These results indicate that there is a common pool of R factors in man and animals.

INTRODUCTION

Although it has long been postulated that R factors in man could be of animal origin (Anderson & Lewis, 1965*a,b*; Anderson, 1968*a,b*), this has been disputed (see, for example, Walton, 1966, 1970, 1975; Smith, 1969). Despite the similarity of many R factors identified in enterobacteria isolated from man and animals, no systematic scrutiny of such R factors has been carried out with the object of establishing the precise degrees of their genetic and molecular relatedness. The identification of several compatibility groups of R factors (Datta & Hedges, 1971; Hedges & Datta, 1971; Grindley, Grindley & Anderson, 1972; Smith, Humphreys & Anderson, 1974) and the establishment of the general rule that plasmids belonging to the same compatibility group have close molecular similarity (Guerry & Falkow, 1971; Grindley, Humphreys & Anderson, 1973) provided methods for determining the precise degree to which R factors (and other bacterial plasmids) of human origin are related to those found in animals. If the groups identified were the same in all species, they had a common origin; if they were dissimilar, they could have arisen independently in man on the one hand and in animals on the other.

METHODS

The plasmids investigated, and their sources, are shown in Table 1.

All the plasmids examined, code for drug resistance, with the exception of the Δ transfer factor of which the identified markers are its own transferability, the mobilization of resistance determinants, its fi^- and I-like nature, and the property of phage restriction in salmonellae and *Escherichia coli* K12 (Anderson & Lewis, 1965*b*; Anderson, 1966, 1968*b*). They belong to compatibility groups already studied genetically by Grindley *et al.* (1972), and in terms of DNA molecular homology by Grindley *et al.* (1973). Each group contained at least one factor of human and one of animal origin.

Seven of the 14 factors were apparently acquired by their carrier strains in England. The remaining seven were from widely separated geographical regions, although three of the

Table 1. The plasmids studied and their origin

Plasmid no.	Compatibility group	Resistances* and other markers	Origin		Phage type	Organism of human or animal origin	Isolated from strain no.
			Organism	Place and date			
240	FII	T, fi ⁺	<i>S. typhimurium</i>	England, 1963	29	Human	3M4466
RJ-19K-		ACSSu, fi ⁻	<i>S. paratyphi</i> B	England, 1964	3a var 4	Human	BB7268
TP166		ACSSu, fi ⁺	<i>E. coli</i>	England, 1971	-	Bovine	EC11321
TP102	I ₁	K, fi ⁺	<i>S. typhimurium</i>	England, 1968	Untypable	Human	8M5645
Δ		-, fi ⁻	<i>S. typhimurium</i>	England, 1964	29	Bovine	RTI
TP120	N	ASSuT	<i>S. typhimurium</i>	England, 1962	1a var. 2	Human	2M1818
TP158		ASSuT	<i>S. typhimurium</i>	Belgium, 1972	Untypable	Bovine	I3M275
TP123	H ₁	CSSuT	<i>S. typhi</i>	Mexico, 1972	Degraded Vi	Human	IT4739
TP153		CSSuT	<i>S. typhi</i>	Vietnam, 1974	E7	Human	IT5479
TP163		CSSuT	<i>S. typhi</i>	Thailand, 1974	53	Human	IT5688
TP171		CSSuT	<i>S. typhimurium</i>	England, 1972	Untypable	Bovine	I2M974
TP154		AKT	<i>S. typhimurium</i>	Singapore, 1973	193	Avian	I3M1653
TP116	H ₂	CSSu	<i>S. typhi</i>	Spain, 1969	C1	Human	IT3616
TP167		CKST	<i>S. typhimurium</i>	Canada, 1974	186	Bovine	I4M10

* Symbols for resistances: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines. All factors resistant to streptomycin were also resistant to spectinomycin, except TP167.

Table 2. *Contour lengths and molecular weights of the plasmids*

Plasmid		Isolated from strain of human (H) or animal (A) origin	No. of molecules measured	Mean contour length $\pm$ s.d. (μ m)	$10^{-6} \times$ Molecular weight* (daltons)
Compatibility group	No.				
FII	240	H	13	19.8 ± 0.3	41.0
	R1-19K ⁻	H	15	26.2 ± 0.5	54.2
	TP166	A	10	26.7 ± 0.6	55.3
I ₁	TP102	H	15	28.5 ± 0.6	59.0
	Δ	A	30	28.7 ± 0.5	59.4
N	TP120	H	11	15.3 ± 0.2	31.7
	TP158	A	12	17.4 ± 0.2	36.1
H ₁	TP123	H	12	59.5 ± 0.8	123.2
	TP153	H	10	55.2 ± 1.6	114.3
	TP163	H	10	55.5 ± 2.3	114.9
	TP171	A	12	58.5 ± 0.7	121.1
	TP154	A	10	61.3 ± 3.3	126.9
H ₂	TP116	H	19	69.4 ± 1.5	143.7
	TP167	A	10	82.2 ± 3.7	170.2

* Calculated on the assumption that 1μ m of DNA = 2.07×10^6 daltons (Lang, 1970).

five H₁ factors came from salmonellae isolated in South East Asia (Anderson, 1975). The carrier strains were identified between 1962 and 1974.

The mutual incompatibility of plasmids within each group, and their compatibility with those of other groups, were established by the techniques of Grindley *et al.* (1972). The molecular properties and relationships were determined by the methods of Grindley *et al.* (1973). The standard carrier strain was KI2F⁻ strain 703, or its nalidixic acid-resistant mutant 14R525.

RESULTS

Molecular size of the plasmids

Table 2 presents the contour lengths of the plasmids as determined by electron microscopy, and the calculated molecular weights. The molecular sizes of members of each group are of the same order, and the factors of human and animal origin in each group conform generally to the characteristic group size. The only possible exception to this was TP167, an H₂ factor from a strain of bovine *S. typhimurium* isolated in Canada. This factor was considerably larger than TP116, the only other H₂ representative studied, but its order of magnitude was closer to TP116 than to any of the factors of other groups. The H₂ plasmids are among the largest so far described.

The difference between the contour length of plasmid 240 (T resistance) and those of R1-19K⁻ and TP166 (ACSSu resistance) is probably caused by the presence of the ACSSu linkage group in the latter two plasmids. Dissociation of the ACSSu region from the complete R1-19K⁻ plasmid in *S. typhimurium* and *E. coli* reduces the length of the plasmid by 7μ m to about 19μ m (Cohen & Miller, 1969; Humphreys, Willshaw and Anderson, unpublished).

DNA reassociation studies

The results shown in Table 3 and Fig. 1 establish clearly the close relationship between the DNA of plasmids of each compatibility group: the homology was always more than 50 %, and usually much higher. In contrast, little homology was evident with plasmids of

Table 3. Homology between plasmids of animal and human origin

The values indicate the degree of reassociation at 75 °C of ³H-labelled plasmid DNA with unlabelled plasmid DNA relative to the reassociation both with DNA of the same plasmid (= 100) and with *E. coli* K12 chromosomal DNA (= 0).

Unlabelled DNA from strains bearing plasmids			Labelled plasmid DNA											
Group	Human (H) or animal (A) origin	Plasmid no.	FII		I ₁		N		H ₁		H ₂			
			240	RI-19K-TP166	TP102	Δ	TP120	TP158	TP123	TP153	TP154	TP116	TP167	
FII	H	240	100	57	14	13	0	0	3	—	4	0	3	
	H	RI-19K-TP166	—	100	—	—	—	—	17	—	—	—	—	
	A	TP166	64	85	9	10	10	—	17	6	5	9	4	
I ₁	H	TP102	13	—	100	75	7	4	2	—	3	—	—	
	A	Δ	15	—	65	100	1	0	—	—	4	0	0	
N	H	TP120	1	—	0	1	100	94	5	—	0	0	2	
	A	TP158	1	—	1	0	101	100	10	—	—	—	—	
H ₁	H	TP123	10	17	0	0	9	6	100	91	91	8	0	
	H	TP153	—	6	—	—	—	3	94	100	95	5	0	
	H	TP163	10	6	1	0	8	4	88	96	82	7	5	
	A	TP171	—	—	—	—	—	—	—	100	—	—	—	
	A	TP154	6	0	0	0	7	12	87	92	100	0	0	
H ₂	H	TP116	2	—	—	0	8	—	4	0	—	100	65	
	A	TP167	—	—	—	—	—	—	—	0	—	81	100	

The broken line encloses reactions of labelled plasmid DNA with unlabelled plasmids from the same compatibility group.

—, Not done.

Fig. 1. Histograms illustrating the results in Table 3. The findings with each labelled plasmid DNA are plotted individually and divided into compatibility groups as follows. (a) Labelled DNA from the group FII plasmids: ■, 240 (human origin); □, R1-19K⁻ (human origin); ▨, TP166 (animal). (b) Labelled DNA from the group I₁ plasmids: ■, TP102 (human); ▨, Δ (animal). (c) Labelled DNA from the group N plasmids: ■, TP120 (human); ▨, TP158 (animal). (d) Labelled DNA from the group H₁ plasmids: ■, TP123 (human); ▨, TP154 (animal). (e) Labelled DNA from the group H₂ plasmids: ■, TP116 (human); ▨, TP167 (animal). Experiments not done are indicated by a star.

heterologous groups, although figures in excess of about 10 %, as found between the FII group with I₁ and H₁ plasmids, may indicate minor degrees of genetic similarity, either in regions determining antibiotic resistance or in other, unidentified, regions. The outstanding feature of these studies, however, was the close correspondence between the nucleotide sequences of the plasmids of human and of animal origin in each compatibility group.

DISCUSSION

The high degree of DNA homology between the auto-transferable plasmids isolated from the enterobacteria of man and of animals establishes unequivocally that in all important respects these plasmids are identical. It can therefore be concluded that they are drawn from a common pool. Differences in genetic markers, such as those for drug resistance, between members of the same group do not affect this argument, because the resistance determinants emerge in response to pressure by a range of antibiotics. Although they recombine with the respective transfer factors to form R factors, they are of independent origin and may indeed represent the only disparity between the DNA of members of a given compatibility group. Moreover, the same resistances are found in man as in animals, in members of each plasmid compatibility group.

The question that naturally arises is: which is the direction of flow of R factors and other plasmids – from animals to man or vice versa? Man must contribute to the plasmids of animals, but the opportunity for flow in the opposite direction is probably much greater, bearing in mind the environmental contamination with animal enterobacteria which results from poor food hygiene, the handling of uncooked meat, and the consumption of inadequately cooked meat products. The continuous occurrence of outbreaks of salmonella food poisoning, in which the salmonellae concerned frequently carry R factors they are known to have acquired in animals, suggests that the animal contribution may be substantial (Anderson & Lewis, 1965*a,b*; Anderson, 1968*a,b*). However, these are special cases, and the extent to which animals are a source of R factors in man cannot easily be determined. Unless plasmid-bearing non-pathogenic enterobacteria such as *E. coli*, of animal origin, can be identified with certainty in man, this problem may remain largely unsolved, since enterobacteria of animal origin, which do not establish themselves in the human intestine, may nevertheless transfer their plasmids to the resident human enterobacteria before disappearing (Anderson, 1968*c*).

A drastic cutback in the use of antibacterial drugs would probably result in a reduction in the size of the reservoir of resistance plasmids. But *E. coli* carrying R factors are present and apparently stable in many persons who have never been treated with antibiotics (Anderson, Gillespie & Richmond, 1973; E. S. Anderson, unpublished). This indicates that once some R factors, from either animal or human sources have gained entry to the enterobacterial population, maintenance of antibiotic pressure is unnecessary for their persistence. Whether total cessation of the use of antibacterial drugs would ultimately result in a massive reduction in the distribution of these plasmids is an unanswerable question, because such cessation cannot be achieved. Indeed, even partial withdrawal is unlikely, though a temporary local withdrawal of the type described by Price & Sleight (1970) and Lowbury, Babb & Roe (1972) can be an effective solution to problems of hospital infection with drug-resistant opportunist pathogens in individual units or areas. Continuous international monitoring of the distribution of R factors and other plasmids in man and animals is necessary, so that information on this vitally important aspect of human and animal ecology is constantly available.

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