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Two Major Groups of Colicin Factors: Their Evolutionary Significance

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Summary. Eleven colicin factors have been placed in two groups defined by a number of physiological criteria such as the effect of the host *recA* (recombination-proficiency) allele on colicin titres and the maximum number of copies of the colicin factor per chromosome. The fundamental difference between the two groups may lie in the molecular weight of the plasmid DNA: one group is about 5×10^6, the other about 70×10^6. The colicins specified by members of each group are also related. Colicin factors within the same group may therefore resemble each other because they are descended from the same ancestral plasmid which was either 'EK-like' of low molecular weight or 'BIV-like' of high molecular weight.

Introduction

The colicin (Col) factors considered here are a class of bacterial plasmids which determine the synthesis of antibacterial proteins named 'colicins' (see Frédéricq, 1963; Reeves, 1972). These factors are conventionally classified in a number of groups designated B, I, K,... by the host range of their colicins on a set of standard colicin-sensitive indicator strains (see Frédéricq, 1965). However, when other attributes of these Col factors are considered, they fall into only two groups. One group has a molecular weight of about 5×10^6, the other of about 70×10^6 (Hardy and Meynell, 1972a; Meynell, 1972). Indeed, the difference in molecular weight may underly all the other differences observed between the two groups. Factors within the same group might resemble each other merely because they had similar molecular weights. However, the same groups become apparent on considering the bacterial loci determining decreased susceptibility to these colicins, which indicates a similarity between the colicin proteins. Factors within each group therefore appear to be related, possibly because they are descended from the same ancestral plasmid (Hardy and Meynell, 1973).

Methods

Culture Media. Nutrient agar was Oxoid Blood Agar Base. Nutrient broth was Oxoid Nutrient Broth No. 2. Salts medium was the citrate-free mixture given in Meynell and Meynell (1970). The supplemented salts medium used for isotopic incorporation is described below.

Colicin Factors. These are listed in Table 1. Some have their own sex factor and are self-transmissible; others do not comprise a sex factor but are co-transferred by the accompanying sex factor I-16. Most of these Col factors are well known, with the exception of ColE1a-16. This is transmissible by its original host (Lewis and Stocker, 1965) because it carries an unlinked sex

factor (Meynell & Lawn, 1968), subsequently named I-16 (Edwards and Meynell, 1969). The colicin determinant has since been separated from the I-16 sex factor by a short interrupted mating, as described below, and the term 'ColE1a-16' will now be used only for this non-transmissible plasmid determining colicin E1a-16.

Bacteria. The majority of strains were derived from *Escherichia coli* strain K-12 and their genetic markers are given by Bachmann (1972). The exceptions are the strains of *Salmonella typhimurium* strain LT2 listed in Table 1, all of which were streptomycin-sensitive and were used as sources of Col factors.

The colicin indicator strain was *Escherichia coli* strain M441, i.e. strain PA309, resistant to streptomycin (200 μg/ml agar), nalidixic acid (100 μg/ml agar) and to phages T1 and T5. Because of its phage-resistance, strain M441 was resistant to colicin M which is formed by many strains forming colicin B (Frédéricq and Šmarda, 1970; Hardy and Neimark, 1972).

The Col$^+$ strains of Table 2 were derivatives of *Escherichia coli* strains J6-2 (*recA*$^+$ *strA*) and M2545 which is a recombination-deficient (*recA*) derivative of J6-2 prepared by Dr. Pearl Cooper by crossing-in the *recA* locus of the Hfr donor, MA1048, of Dubnau and Maas (1968). Before introducing colicin factors, each strain was made resistant to the colicin concerned, as shown by the plate receptor test of Hill and Holland (1967).

The Col factors were introduced into the colicin-resistant derivatives of strains J6-2 and M2545 by conjugation as follows. Each mating mixture consisted of 10 vol. of a growing broth culture of the Col$^+$ *str-s* donor culture (Table 1) at 10^8/ml added to 1 vol. of an overnight culture of the *str-r* recipient. The mixture was left at 37° either for 3 hr when the donor's sex factor was continually de-repressed or for 3 hr followed by dilution 1/10 in fresh broth and incubation overnight when the sex factor was repressible. Col$^+$ recipient clones were isolated from the mating mixture by plating dilutions in an overlay of 2.5 ml soft agar (0.75%, w/v, agar) containing streptomycin (200 μg/ml) which was poured on a plate of 25 ml nutrient agar also containing streptomycin, 200 μg/ml, to inhibit the donor. A second overlay of 2.5 ml soft agar was poured on top of the first and the plate incubated at 37° for one night for E and K colicins or two nights for B and I colicins. Supplemented glucose-salts agar was used with ColV factors and was incubated for two nights in order to obtain distinct inhibition zones. A third layer of 2.5 ml soft agar inoculated with the indicator (10^8/ml) was then added and the plates incubated for a further night at 37°. Col$^+$ clones of the recipient could then be recognized by the overlying inhibition zone in the layer of indicator.

Both Helinski and Herschman (1967) and Kennedy (1971) stated that Col$^+$ derivatives of *recA* strains could not be detected by their double-overlay techniques and had to be identified by random testing of recipient clones for immunity to colicin. However, in our hands, although *recA* recipients gave smaller zones, the above method readily detected all Col$^+$ recipients, wheter *recA* or *recA*$^+$.

The Col$^+$ strains used in molecular weight determinations were derived from CL145 (i. e. 58–161 resistant to E colicins and to streptomycin) for ColE1a-16; KH324 (i. e. J6-2 resistant to K colicins and to nalidixic acid) for ColK-235; and KH252 (i.e. AB1157 made resistant to V colicins) for ColV-K30 and ColV-K94. ColE1a-16 was introduced alone into strain CL145 by interupting mating 2.5 min after adding the donor, according to Smith, Ozeki and Stocker (1963). Interupted matings were similarly used to introduce ColK-235 alone into strain KH324, and ColV-K30 into strain KH252 (since strain K30, the donor of ColV-K30, also carries ColE1).

Colicin Titrations. These were done by the drop method (see Meynell and Meynell, 1970). Cultures were grown in nutrient broth to an optical density of 1.0 (Hilger 'Spekker' photometer, 1 cm cuvettes, red filter) and samples treated with ultrasonics (Dowman and Meynell, 1970; Hardy and Meynell, 1972c). The treated sample was then diluted in two-fold steps, using a fresh pipette for each step to avoid falsely high titres, in a mixture of 95 vol 0.1 M phosphate buffer, pH 7.0, and 5 vol nutrient broth. One drop of 0.02 ml was distributed from each dilution by a calibrated dropping pipette on a soft agar overlay previously inoculated with the indicator strain, M441. Both overlay and base agar contained nalidixic acid, 100 μg/ml, to inhibit surviving Col$^+$ bacteria. Plates were incubated at 37° overnight, and the colicin titre taken as the smallest concentration detectably inhibiting the indicator.

'Free' colicin is that colicin remaining in the supernatant of cultures centrifuged for 20 min at 2,000 r.p.m. in a bench angle centrifuge (M.S.E. model 50). 'Bound' colicin was

measured by resuspending the deposited cells in buffer to the original volume of the culture and by treating with ultrasonics before titration. 'Total' colicin is the sum of free and bound colicin: if their titres were respectively 32 and 256, the total is 288. 'Mean' colicin titres in Fig. 1 are the arithmetic means of three independent cultures: if the individual titres were 32, 64 and 64, their mean is 53.

Lacuna Counts. 0.5 ml chloroform was added to 4.5 ml culture (Ozeki, Stocker and de Margerie, 1959), agitated on a mechanical mixer and incubated for 25 min at 37°. 1 ml of an appropriate dilution was mixed with 10^8 cells of the indicator strain and 2 ml soft agar at 48°, and poured on a plate of nutrient agar. Both overlay and base agar contained nalidixic acid (100 μg/ml).

Molecular Weights of Col Factor DNA. The molecular weights of ColE1a-16 and ColK-235 were determined by sedimentation of their ^{3}H-labelled DNA through preformed 5–20% sucrose gradients (pH 8), using as an internal standard ^{14}C-labelled ColE2-P9 DNA whose molecular weight is known to be 5×10^6 (Bazaral and Helinski, 1968). Each strain was grown in salts medium, pH 6.8, supplemented with glucose (4 mg/ml), tryptophan and cysteine (50 μg/ml each), Oxoid acid-hydrolysed casein and Difco yeast extract (5 mg/ml each). ^{3}H-methyl or ^{14}C-labelled thymidine (10 and 2.5 μCi/ml, respectively) and uridine (250 μg/ml) were also present. Cultures in 30 ml medium in 250 ml conical flasks were grown with shaking at 37° for at least 4 generations in labelled medium until the optical density reached approx. 0.3. The cells were then washed twice with saline and resuspended in 1.5 ml saline in a 5 ml cellulose nitrate centrifuge tube (Spinco 305050). 2.5 ml tris-EDTA-saline, pH 8.0, (TES4) saturated with *n*-dodecylamine (Meynell, 1971) and 0.5 ml lysozyme (10 mg/ml TES4) were added, and the tube incubated in a 37° bath for 10 min. Two aliquots, each of 0.25 ml, of sarkosyl (sodium lauroyl sarkosinate, 5%, w/v, in TES4) were injected into the suspension which usually cleared almost immediately. The tubes were next centrifuged at 20° for 35 min at 30000 r.p.m. in a swing-out rotor (M.S.E. 3×5 ml) to give a 'cleared lysate' from which most of the chromosomal but little of the plasmid DNA had been sedimented (Clewell and Helinski, 1969; Hardy and Meynell, 1972b). The upper 1 ml supernatant was saved from each tube. The ^{3}H-labelled and ^{14}C-labelled supernatants were mixed and 0.2–0.3 ml of the mixture layered on the surface of a 5–20% (w/v) sucrose gradient prepared in TES4. This was centrifuged in the same rotor at 20° for 4.2 hr at 35000 r.p.m., the time calculated to bring the ColE2-P9 standard to the midpoint of the gradient (McEwen, 1967). The gradients were analysed by piercing the base of each tube and by collecting drops on filter paper discs which were washed successively in trichloracetic acid, ethanol and acetone before being counted in a scintillation spectrophotometer.

The molecular weights of ColV-K30 and ColV-K94 were estimated by sedimentation through preformed 5–20% alkaline sucrose gradients prepared in 0.3 N NaOH, using isotopically-labelled cells lysed with alkaline sarkosyl (Freifelder, Folkmanis and Kirschner, 1971. For details, see Spratt, Rowbury and Meynell, 1973). An R factor of known molecular weight (R1*drd*19, mol. wt. 65×10^6: Silver and Falkow, 1970) was used as an internal standard.

Results

The present investigations stemmed from a systematic examination of two physiological characteristics of eleven colicin factors. First, the proportion of total colicin bound to the cells was measured, since Dowman and Meynell (1970) had found unexpectedly that colicin Ib-P9 was almost entirely bound. Second, the effect on colicin titres of the host *recA* (recombination-proficiency) allele was examined, since reports by Ben-Gurion (1967), Helinski and Herschman (1967) and MacPhee (1970) suggested that the titres of some colicins like E2-P9 were markedly diminished in *recA* strains whereas titres of others like Ib-P9 were not affected. After the results of these tests had been analysed, they were seen to be correlated with the molecular weight of the Col factor DNA, where this was known (Meynell, 1972). However, no values had been published for ColE1a-16, ColK-235 or the ColV factors, and these were therefore determined.

Table 1. Colicin factors

Colicin factor	From	Transferred by sex factor	Reference
B-K77	*E. coli* K77	Own	Frydman and Meynell (1969)
B-K98	*E. coli* K98	Own	Frydman and Meynell (1969)
B-K166	*E. coli* K166	Own	Frydman and Meynell (1969)
E1a-16	*Salm. typhimurium* M626	I-16	Lewis and Stocker (1965)
E1-K30	*Salm. typhimurium* M543	I-16 *drd*	Meynell and Lawn (1967, 1968)
E2-P9	*Salm. typhimurium* SL636	I-16 *drd*	Meynell and Lawn (1967, 1968)
Ia-CT4	*E. coli* M498	Own	Stocker (1966)
Ib-P9	*Salm. typhimurium* SL1066	Own	Stocker (1966)
K-235	*Salm. typhimurium* SL711	I-16 *drd*	Lewis and Stocker (1965)
V-K30	*E. coli* K30	Own	Macfarran and Clowes (1967)
V-K94	*E. coli* M2600	Own	Macfarran and Clowes (1967)

Table 2. Titres of bound and free colicin spontaneously produced by *Escherichia coli* J6-2 (*recA*$^+$) and M2545 (*recA*)

Colicin factor	Bound colicin *recA*$^+$/*recA* Titration:			Free colicin *recA*$^+$/*recA* Titration		
	1.	2.	3.	1.	2.	3.
B-K77	32/8	64/8	64/16	8/<1	4/<1	8/<1
B-K98	1024/32	256/32	128/16	16/<1	32/<1	8/<1
B-K166	128/16	256/16	256/16	8/<1	8/<1	16/<1
E1a-16	256/8	128/8	512/16	256/4	256/2	256/2
E1-K30	128/8	128/8	256/16	512/4	512/4	1024/4
E2-P9	256/16	256/8	512/16	2048/4	2048/4	1024/4
Ia-CT4	32/8	16/8	32/16	2/<1	2/<1	2/<1
Ib-P9	128/64	128/64	128/32	4/<1	2/<1	4/<1
K-235	256/16	1024/32	1024/32	2048/4	1024/2	1024/2
V-K30	8/8	8/8	8/16	1/<1	1/<1	1/<1
V-K94	8/8	16/8	8/8	1/<1	1/<1	1/<1

Each entry is the titre by drop method; i.e. the highest dilution of colicin inhibiting the indicator. Bound and free colicin (see Materials and Methods) were titrated on three independent cultures of each strain. The values for each culture appear at corresponding points in the Table. Thus, the first titration of bound and free colicin B-K77 in the *recA*$^+$ host gave titres of 32 and 8, respectively.

Comparative Features of Colicin Synthesis

Bound and Free Colicin. The titres of bound and free colicin were determined by the drop method for each of the eleven Col factors listed in Table 1. Each titration was performed three times on independent cultures to yield the titres given in Table 2.

There were two main findings:

(a) In the *recA*$^+$ host, the ratio of bound/free colicin differed greatly for individual factors (Hardy and Meynell, 1972a), as can be seen from the mean ratios

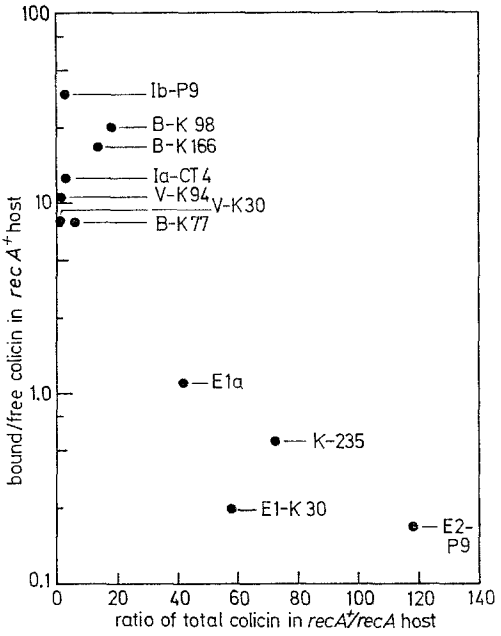

Fig. 1. Comparative features of colicin synthesis. Ordinate: mean values for bound/free colicin in *recA*+hosts. Abscissa: ratio of total colicin in *recA*+/*recA* hosts. Mean and total colicin calculated from the entries of Table 2 as described in the Methods

plotted in Fig. 1. Thus, colicin B-K77 resembled Ib-P9 in being almost entirely bound, whereas colicin K-235 resembled E2-P9 in being almost entirely free.

In the *recA* host, however, nearly all the colicin was bound, whichever Col factor was examined.

(b) Colicin titres in the *recA*+ host were usually less with the transmissible factors of the B, I and V groups than with the non-transmissible factors of the E and K groups (Fig. 1).

In the *recA* host, differences in titre were negligible.

Effect of the Host recA Allele on Colicin Titres. Marked differences between individual Col factors became evident while preparing Col+ derivatives of the *recA* (recombination-defective) strain since the inhibition zones formed by members of the E and K groups were noticeably smaller than in the *recA*+ host. These differences were confirmed by the colicin titrations whose results are given in Table 2. The mean total colicin titre in *recA*+/*recA* hosts is plotted in Fig. 1. They show that titres of colicins like E2-P9 and K-235 were more than sixty times greater in the *recA*+ host, whereas titres of others like the I and V colicins were essentially the same in both hosts.

Molecular Weights of Colicin Factors

Non-Transmissible Factors. These molecular weights were estimated by sedimentation through preformed sucrose gradients (pH 8), using as internal

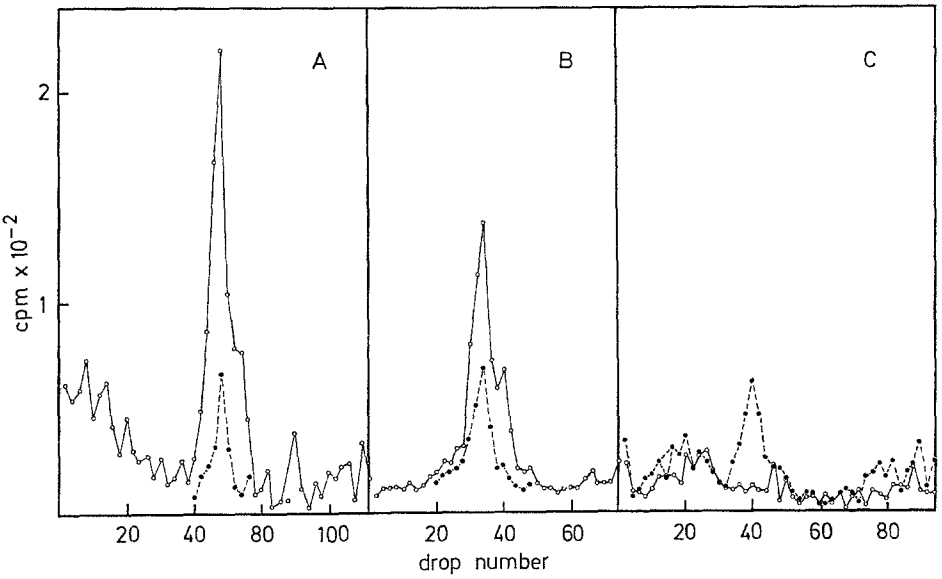

Fig. 2 A—C. Sedimentation of cleared lysates containing ColK-235 or ColE1a-16 through 5–20% sucrose gradients, pH 8. Ordinate: c.p.m. Abscissa: drop number. The top of each gradient is on the right. • ---• ColE2-P9 standard. A) ColK-235, B) ColE1a-16. C) strain CL145 Col$^-$

standard ColE2-P9 whose molecular weight is 5×10^6 (Bazaral and Helinski, 1968).

The ColE2-P9 standard was found approximately in its expected position, in the middle of the gradient. The Col$^-$ parental strains gave either no peak or a variable diffuse band, presumed to be chromosomal fragments, which was slightly in advance of the standard and which was estimated to be approx. 40 S (Fig. 2C). The strain carrying ColE1a-16 yielded a prominent peak at the same position as ColE2-P9 which was assumed to be the closed-circular monomer, and a second smaller peak of about 19S which may have been composed of relaxed circular monomers produced by the sarkosyl (Clewell and Helinski, 1969). The strain carrying ColK-235 behaved qualitatively in the same way but the second minor peak was less pronounced (Fig. 2A, B). Both ColE1a-16 and ColK-235 therefore appear to be about the same size as CollE2-P9: namely, of molecular weight 5×10^6.

This estimated molecular weight implies that each Col factor is about 0.2% of the size of the chromosome of *Escherichia coli* K-12. However, in three independent experiments, the counts on Col factor DNA recovered in the gradients, expressed as a percentage of the total counts before the clearing spin, were 0.78%, 1.25% and 1.38% for ColE1a-16, and 0.73%, 0.89% and 1.35% for ColK-235. All these values are considerably in excess of 0.2% so that there must have been at least 3.5–6.9 copies per chromosome without allowing for losses in the clearing spin. These Col factors, like ColE1-K30 (Bazaral and Helinski, 1970) and ColE2-P9

(Hardy and Meynell, 1972b), which are of similar size, therefore appear to replicate under relaxed control.

Transmissible Factors. These molecular weights were estimated by sedimentation through alkaline sucrose gradients (0.3N NaOH), using as internal standard the R factor, R1*drd*19, whose molecular weight is 65×10^6 (Silver and Falkow, 1970).

The Col$^-$ strain yielded no peak in the gradient. ColV-K94 sedimented more rapidly than the standard (Fig. 3A), and ColV-K30 sedimented more rapidly than ColV-K94 (Fig. 3B). The molecular weights of both Col factors therefore exceeded 65×10^6 and were estimated at about 80×10^6. Clowes (1972, Fig. 18) reported a value of 94×10^6 for ColV-K94.

Only a small percentage of the total DNA was found in the closed circular plasmid forming the peaks in the gradients. The highest recovery from four gradients was 0.45% for ColV-K30 and 0.67% for ColV-K94. Given the estimated molecular weight, these recoveries correspond to only 0.1–0.2 copies of the Col factor per chromosome. However, other workers have reported about 1 copy per chromosome (Hausmann and Clowes, 1971).

Discussion

Comparative Features of Colicin Synthesis

Bound and Free Colicin. The ratio of bound/free colicin presumably reflects the manner in which the colicin is synthesized and released from the cells. Similar observations on the kinetics of extracellular enzyme synthesis lead to a straightforward conclusion (Collins, 1964): when the ratio is low, the colicin is released almost as soon as it is synthesized; when it is high, the colicin remains stably associated with the organisms for several generations.

Effect of the Bacterial recA Allele. On considering the ColE and ColK factors, the lower titres of their colicins produced in recombination-deficient (*recA*) hosts recalls that the spontaneous induction of vegetative growth of many temperate phages is diminished in *recA* lysogens (Brooks & Clark, 1967; Hertman and Luria, 1967; Wing, 1968) and that colicin factors have frequently been likened to phages (Jacob and Wollman, 1961, p. 319; Frédéricq, 1963). Conceivably, therefore, the ColE and ColK factors become 'induced' spontaneously in occasional cells of the culture which, as a result, begin to synthesize colicin. The effect of the *recA* locus might then be to lower the proportion of spontaneously induced cells. This interpretation is consistent with only a proportion of *recA*$^+$ cells forming lacunae (Ozeki *et al.*, 1959; Kennedy, 1971; Hardy and Meynell, 1972d) and with this proportion being less when the host is *recA* (Kennedy, 1971; Hardy, unpublished). Moreover, agents like mitomycin-C cause up to 100% of cells to form lacunae, with a concomitant increase in colicin titres, which might reflect induced vegetative replication of the Col factor. DeWitt and Helinski (1965) reported that, following exposure of *Proteus mirabilis* carrying ColE1-K30 to mitomycin, the amount of Col factor DNA increased in parallel with the colicin titre. However, neither Inselburg (1970), using K-12 mini-cells carrying ColE1-K30, nor Hardy and Meynell (1972b), using K-12 bacteria carrying ColE2-P9, found any specific increase in Col factor DNA after exposure to mitomycin-C.

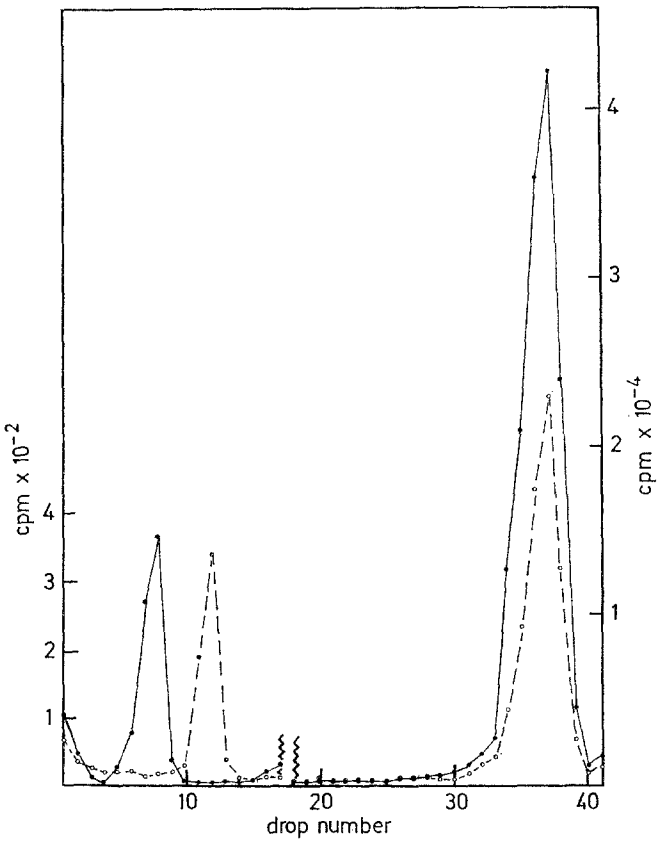

Fig. 3A

Fig. 3A and B. Sedimentation of whole bacterial lysates containing ColV-K30 or ColV-K94 through alkaline 5–20% sucrose gradients, ca. pH 13. Ordinate- c.p.m. (note changes in scale). Abscissa: drop number. The top of each gradient is on the right. A) • --- • ColV-K94. o --- o R1*drd*19 standard. B) • --- • ColV-K30. o --- o ColV-K94. The arrow marks the position of the R1*drd*19 standard

The latter observations are not necessarily inconsistent with these factors undergoing vegetative replication like phage. Under the conditions of such experiments, each cell contains many copies (e.g. 20–40). If each copy has only a small independent probability of being expressed in untreated (Hardy and Meynell, 1972d) and in mitomycin-treated cultures, as our preliminary results suggest, then the majority of uninduced copies in each cell may mask the behaviour of the minority that is induced.

On considering the ColB, I and V factors, the host *recA* allele might not affect expression of the colicin determinant for several reasons:

(a) Expression is not dependent on a RecA product determined by either bacterium or plasmid. That is to say, colicin synthesis is not a consequence of any form of RecA-dependent 'induction' affecting the plasmid as a whole and

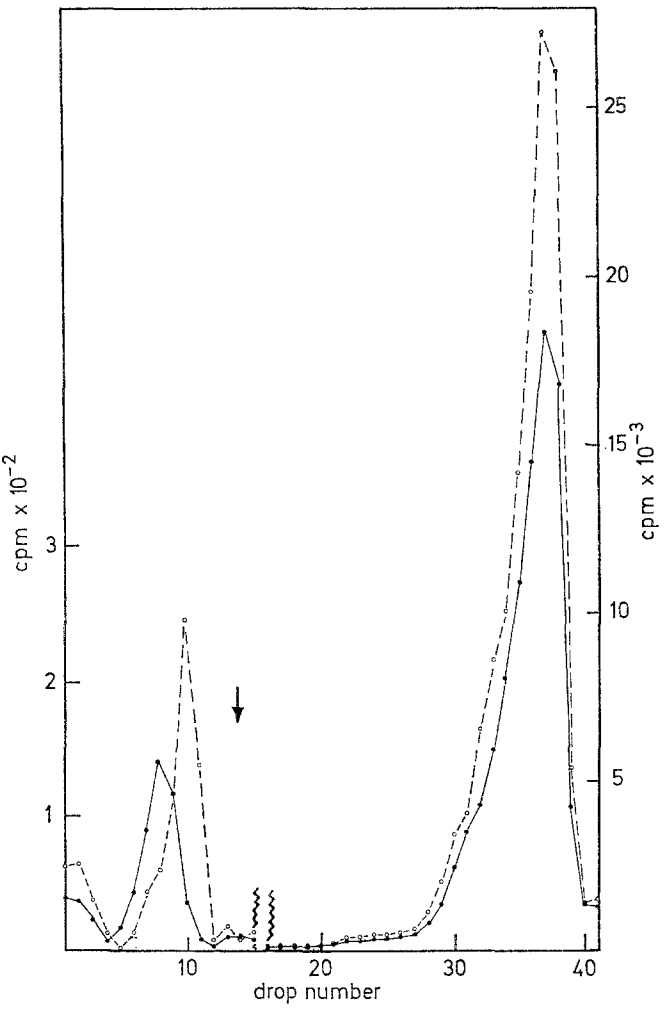

Fig. 3B

the colicin determinant is expressed independently of the rest of the Col factor. A factor like ColIb-P9 with its I sex factor and its linked *col* gene may be analogous to *Flac* with its F sex factor and its linked lactose region which form independent units of expression. The *col* gene might therefore be either repressed or constitutive, and colicinogeny determined by these factors might be analogous to phage conversion (Hardy and Meynell, 1972a);

(b) Expression does in fact depend on a RecA product but this is not apparent because either:

(i) The RecA product is supplied by the Col factor so that colicin titres in a *recA* host are as high as in one that is *recA*$^+$. However, no RecA function is demonstrable in ColIb-P9 inasmuch as no more colicin E2-P9 is produced by a *recA*

ColE2$^+$ strain after acquiring ColIb-P9, and mitomycin-C fails to increase colicin titres in *recA* hosts carrying ColB, I or V factors (Hardy and Meynell, 1972c); or

(ii) The ColB, I and V factors cannot respond to the RecA product of *Escherichia coli* K-12 because they originated in a functionally unrelated host. Thus, titres in *recA*$^+$ K-12 would be as low as in a *recA* strain. This possibility was suggested by the titres of this group, whether in *recA*$^+$ or in *recA* hosts, being of the same order as the titres produced by ColE and K factors in *recA* hosts.

Table 3. Characteristics distinguishing two groups of colicin factors

	Group I	Group II	Source
Members	E1, E1a, E2, K	B, I, V	Fig. 1
Colicin	Mostly free	Mostly bound	Table 2
Colicin titres in:			
recA host	Decreased markedly	Decreased little, if at all	Table 2
recA$^+$ host	~512	~16	Table 2
Lacunae	Unambiguous	Faint or absent	Unpublished
Self-transmissible	No	Yes	Meynell, Meynell and Datta (1968); Meynell (1972)
Plasmid mol. wt.	$\sim 5 \times 10^6$	$\sim 70 \times 10^6$	Bazaral and Helinski (1968); Clewell and Helinski (1970); Hausmann and Clowes (1971); Meynell (1972); Clowes (1972)
Maximum number of copies per chromosome	$\gg 1$	~1	Bazaral and Helinski (1970); Hausmann and Clowes (1971); Clewell (1972); Meynell (1972); Clowes (1972)
Plasmid DNA binding to Cd-sarkosyl crystals	30%	70%	Dowman and Meynell (1973)
Replication dependent on host:			
polA1 locus	Yes[a]	No[a]	Kingsbury and Helinski (1970)
dnaE locus	No	Yes	Goebel (1972); Goebel and Schrempf (1972)

[a] Factors tested: E1-K30, E2-P9, Ib-P9, V-K30. For the effect of the host *dnaA* locus, see Goebel (1973).

Two Phenotypic Classes of Colicin Factors

The two criteria, the ratio of bound/free colicin in *recA*$^+$ hosts and the effect of the host *recA* allele on total colicin titres, served to place the eleven factors in two groups (Fig. 1). In Group I, comprising the three ColE factors and ColK-235, at least half the colicin was free and titres of total colicin were far less in the *recA* host. In Group II, comprising the ColB, I and V factors, nearly all the colicin was bound and titres were essentially the same in both *recA*$^+$ and *recA* hosts.

On reviewing these two groups, it became clear that they also differed in a number of other important characteristics, including molecular weight of the plasmid DNA and its mode of replication. These differences are listed in Table 3.

The question arises as to whether all the differences observed between the two groups of factors are independent or are merely alternative expressions of a single basic property. The latter seems highly probable and it is likely that all the differences listed in Table 3 arise from the gross difference in molecular weight of the plasmid DNA:

(a) Group I factors are necessarily non-transmissible because they are too small to include the minimum of some ten genes required for sex factor activity (Ohtsubo, 1970; Ippen-Ihler, Achtman and Willetts, 1972);

(b) Numerous copies of a plasmid per bacterial chromosome are found with plasmide of relatively low molecular weight like those of Group I as opposed to those of Group II, notably when exponential growth of the culture has finished. That is, Group I replicates under relaxed, and Group II under stringent, control (see Meynell, 1972; Clowes, 1972). It may be that these plasmids are too small to include genes that recognize the machanism controlling chromosomal replication, although the number of copies of ColE1-K30 and of ColE2-P9 are apparently under some system of control (Clewell, 1972; Hardy and Meynell, 1972d);

(c) Because many copies of Group I factors are present, some may not be membrane-bound, which would account for their lower binding to crystals of cadmium lauroyl sarkosinate (Dowman and Meynell, 1973);

(d) The greater number of copies of Group I factors than of Group II that is found in *recA*$^+$ hosts may explain why the titres of Group I colicins are higher (Hardy and Meynell, 1972d).

(e) The ratio of bound/free colicin seems to depend on the total colicin in the culture. Thus, although the ratio is far lower for Group I than for Group II in *recA*$^+$ hosts, the ratio is approximately equal (and > 1) when Group I are in *recA* hosts; i.e. when the total colicin titre is approximately the same as that associated with Group II. It could be argued that a bacterial cell has a limited capacity for carrying colicin in the bound state and that, when this capacity is fulfilled, the excess colicin becomes free. Such a mechanism, proposed by Jesaitis (1970), would be consistent with the isolation of certain colicins as protein-lipopolysaccharide complexes from untreated cultures but as simple proteins from the same strains exposed to mitomycin-C (Jesaitis, 1970). Thus, the low ratio of bound/free colicin formed by Group I factors in *recA*$^+$ hosts might simply reflect higher colicin titres;

(f) Lacunae produced by strains harbouring Group I factors are large and distinct, whereas those produced by those carrying Group I factors are barely detectable and may be absent. This difference may reflect the greater titres of colicin and lower ratio of bound/free colicin characteristic of Goup I factors in *recA*$^+$ hosts.

Natural Significance of the Two Groups

The existence of these groups can be interpreted in two ways.

First, that all colicin factors arose independently and that some unknown process of natural selection has brought about a situation where they have come

to form two groups. Thus, the B, E, I, K and V factors might be descended from five ancestral B, E, I, K and V plasmids, respectively.

Second, that these colicin factors did not arise independently and, despite the considerable diversity within each of the main groups noted below, there are only two groups because there were only two ancestral col factors: an 'EK' ancestor of low molecular weight and a 'BIV' ancestor of high molecular weight (Hardy and Meynell, 1973). A parallel is provided by the plasmid-borne genes determining sex pili in *Escherichia coli* and *Salmonella*, many of which are unequivocally 'F-like' or 'I-like', despite differences within each group revealed by serological analysis and by susceptibility to donor-specific phages (Lawn and Meynell, 1970; Meynell, 1972).

The second possibility, a common ancestry for each group, is favoured by the finding that the Col factors within each group are also related by the chromosomal loci determining decreased bacterial susceptibility to their colicins. Tolerance to Group I colicins (E1, E2, E3 and K) is determined by the locus *tolAB* placed at 17 min on the standard map of *Escherichia coli* K-12; whereas resistance to Group II colicins (B, I and V) is determined by an unrelated locus, *tonB*, placed at 25 min (Taylor and Trotter, 1972). At the taxonomic level of the groups, it is nevertheless clear that some of the more conspicuous features of these Col factors may not be reliable guides to their degree of similarity in terms of the base sequence homology of their DNA. In Group I, although colicins E2-P9 and E3-CA38 exert completely different biochemical effects, some 80% of their DNAs will hybridize (Inselburg, 1973). In group II, the sex factors differ insofar as those of ColB and ColV are F-like whereas that of ColI is I-like and is completely dissimilar, as far as is known (see Meynell, 1972). However, the sex factor region may have a molecular weight of 15×10^6 or less, and so give little information about the remainder of these plasmids.

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