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The sex factor of colicin factor E1a

By G. G. MEYNELL and A. M. LAWN

*Guinness-Lister Research Unit and Department of Electron Microscopy, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.*1

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Many bacterial plasmids, including colicin factors, enable their host cells to conjugate with recipient strains, to whom the plasmid is then transmitted. Such plasmids therefore possess a sex factor determining synthesis of the sex pilus and other functions concerned in transmission, in addition to genes such as those controlling colicin production. The properties of sex pili suggest that the majority of transmissible plasmids of the *Enterobacteriaceae* possess a sex factor related either to F or to the sex factor of colicin factor Ib, here termed 'Ib' (Lawn, Meynell, Meynell & Datta, 1967). Further support for this view comes from an examination of *colE1a*, which determines the synthesis of colicin E1a, unrelated to colicin I. Lewis & Stocker (1965) found that *colE1a*, unlike *colE1b* or *colE1-30*, could spread through *col*$^-$ cultures to give high-frequency transfer (HFT) preparations like those already described for *colI* by Stocker, Smith & Ozeki (1963). This observation suggested that *colE1a* might consist of the determinants of colicin E1a associated with Ib.

Two *E1a* factors were used in various mutant lines of *Salmonella typhimurium* strain LT2: *E1a-16* from strain SL1016 and *E1a-18* from strain SL1018, originally identified by Lewis and Stocker in strains 1M48 and 1M349, respectively, of the Enteric Reference Laboratory, Colindale. Both factors underwent epidemic spread through *col*$^-$ cultures, as found by Lewis & Stocker (1965). However, when the recipient culture carried *colIa-CA53* or *colIb-P9*, a proportion of recipient cells acquired *colE1a-16* but this did not subsequently spread (Table 1). In the reverse cross, *colIb-P9* × *colE1a-16* or *-18*, some recipient cells

Table 1. *Behaviour of donated* col *factors in* col$^-$ *and* col$^+$ *recipient strains*

Donor's *col* factor	Recipient's *col* factor	Relative numbers of *col*$^+$ recipients after 20 min.	% recipient *col*$^+$ After 20 min.	% recipient *col*$^+$ After growth overnight in streptomycin broth
E1a-16	—	1·0	0·22	8·2
	Ia-CA53	0·27	0·06	0·1
	Ib-P9	0·04	0·0088	0·0062
Ib-P9	—	1·0	1·86	96·0
	E1a-16	0·0003	0·00062	0·00081
	E1a-18	0·01	0·019	0·039

The donor cultures were conventional HFT preparations of streptomycin-sensitive (*str-s*) strains (Stocker *et al.*, 1963). The recipients were *str-r* and could therefore be selected on agar containing 200 μg. streptomycin/ml. Mating mixtures contained about 2×10^7 donor and 2×10^8 recipient cells/ml. broth, and, after 20 min. at 37°C., were diluted 1/20 into broth containing 200 μg. streptomycin/ml. to prevent further transfer from the *str-s* donor strain during subsequent growth.

similarly acquired the donor's *col* factor which again failed to spread as in a *col*$^-$ recipient. The interpretation of these findings is as follows. The majority of established *colI*$^+$ (Ozeki, Stocker & Smith, 1962) or *colE1a*$^+$ cells are incapable of transmitting their *col* factor at any one time because, shortly after it is acquired, its sex factor becomes repressed. When such cells are used as recipients, any donated *col* factor they receive is therefore immediately exposed to repressor and, if its sex factor is susceptible, it is at once repressed, so preventing its epidemic spread. Table 1 therefore suggests that *colE1a* is susceptible to the *colI* repressor and vice versa. It has often been suggested that the small proportion of donors in established *col*$^+$ cultures might be due to repression (Clark & Adelberg, 1962; Monk & Clowes, 1964*b*; Ozeki, 1965; Meynell & Lawn, 1967), but the behaviour of *colE1a* and *colI*, which are presumably in *trans*, strongly suggests that their donor ability is indeed controlled by a cytoplasmic repressor like that postulated for other systems (Jacob & Monod, 1961). This is distinct from the F repressor, as neither *colI* (Monk & Clowes, 1964*a*) nor *colE1a-16*, *18* repress F in a *col*$^+$ *F*$^+$ strain; i.e. both factors are *fi*$^-$ (Watanabe, 1963).

Recipient clones expressing the donated factor were isolated from the first set of crosses in Table 1 and found to be doubly colicinogenic, showing that the donated factor could replicate in synchrony with its new host. *ColE1a* and *colI* therefore differ in this respect because in the crosses, *Ia* × *Ib* and *Ib* × *Ia*, the donor's factor is never expressed by the recipient. In these crosses, conjugation nevertheless occurs, since *colE2* (which is otherwise non-transmissible) is transferred to *col*$^+$ recipients by donors carrying *colE2* as well as *colI*, as previously shown for *Ib* × *Ib* crosses by Smith, Ozeki & Stocker (1963).

Samples of HFT cultures carrying *colE1a-16* were examined by electron microscopy after exposure to antisera specific for various types of sex pilus followed by negative staining with uranyl acetate (Lawn, 1967). Pili were seen which reacted with antiserum to the pili of the *fi*$^-$ R factor, R144, which forms an I-like sex pilus (Lawn *et al.*, 1967). These pili were not seen in *col*$^-$ cultures or in LFT cultures, and were therefore considered to be sex pili determined by *colE1a-16*.

HFT cultures of both *colE1a* strains were prepared and infected with the filamentous phage, If1, isolated by Meynell & Lawn (in preparation), which attacks cells forming sex pili of the type determined by *colIb* but not those with F-type sex pili (Lawn *et al.*, 1967). Infective centres were first titrated 15 min. after adding phage, before lysis began, and again 105 min. later. During the interval, the number of infective centres increased about 1000-fold in the HFT cultures, whereas no increase occurred in cultures of *col*$^-$ strains. The F-specific phages, MS2 and M13, failed to replicate in these cultures.

SUMMARY

The sex factor of *colE1a* appears related to Ib, the sex factor of *colIb*, by each of three criteria: mutual inhibition of epidemic spread, antigenic structure of the sex pilus and susceptibility to I phage. The failure of each factor to spread in cultures carrying the other implies that donor ability is subject to a cytoplasmic repressor. Unlike two *colI* factors, *colE1a* and *colIa* (or *Ib*) can co-exist to give a doubly colicinogenic strain.

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