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Exclusion, superinfection immunity and abortive recombinants in I+ × I+ bacterial crosses

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The bacterial sex factor, F, is known to be transferred less efficiently to F+ or Hfr cultures than to F− strains (Scaife & Gross, 1962) although this effect can be overcome by starving the recipient to produce an 'F− phenocopy' (Lederberg, Cavalli & Lederberg, 1952; Echols, 1963). Even after transfer, F cannot replicate autonomously in an F+ or Hfr recipient and is believed to be diluted out during subsequent bacterial divisions just as superinfecting phage segregates in a strain rendered immune by lysogenization by a related phage. The first phenomenon may therefore be termed 'exclusion' and the second, 'superinfection immunity' (see Meynell, Meynell & Datta, 1968). An F− phenocopy does not exclude donated F but still exhibits superinfection immunity, just as a non-excluding mutant of phage P22 no longer excludes superinfecting phage although it confers immunity (Walsh & Meynell, 1967; Rao, 1968). Exclusion and superinfection immunity are also evident in crosses mediated by the I sex factor found in plasmids like ColI, ColEIa and *fi*− R factors (Lawn, Meynell, Meynell & Datta, 1967; Meynell *et al.* 1968). On crossing an F*lac* donor with a *lac*− F+ or Hfr recipient, transfer of F*lac* is detected by the appearance of *lac*+ sectors in the recipient colonies, following recombination between the plasmid and host chromosome (Dubnau & Maas, 1968). However, the I factor apparently cannot recombine with the bacterial chromosome (Meynell & Edwards, 1968, and in preparation), so that its presence in the recipient has been demonstrated here by the appearance of abortive recombinants.

In preliminary experiments with the wild-type I factors of ColIa-CA53 and ColIb-P9 in various strains of *Salmonella typhimurium* LT2, the frequency of conjugation was estimated by introducing ColE2 into the donor. This factor is non-transmissible alone but is transferred with I in I+ × I+ crosses (Smith, Ozeki & Stocker, 1963) so that its rate of transfer provides a minimum estimate of the rate of conjugation. High-frequency transfer preparations (Stocker, Smith & Ozeki, 1963), with ColIa (or Ib) and ColE2 in the streptomycin-sensitive donor and intermediate strains, respectively, were mated for 20 min with a Col− streptomycin-resistant recipient and with the same recipient carrying ColIa or Ib, which was then isolated by plating on streptomycin-agar. These crosses showed that the recipient never expressed the donor's ColI factor but did acquire ColE2, although the rate of transfer was less when the recipient also carried ColI. The relative rates of ColE2 transfer from a ColIa donor to ColIa and ColIb recipients compared with a Col− recipient were approximately 0·1 and 0·2; and from a ColIb donor, 0·2 and 0·01. Thus, exclusion was most marked when donor and recipient carried the same ColI factor. However, when the ColI+ recipient was starved by being shaken for 2–4 h in 0·1 M phosphate buffer the rate of ColE2 transfer was almost equal to that observed with a Col− recipient. The recipient ability of Col− strains was unaffected by starvation. Exclusion by an I factor was therefore removable by starvation, to produce an I− phenocopy.

Since the donor's ColI factor was not expressed by ColI+ recipients there was no

evidence it had been transferred. A different donor was then used which carried the complex plasmid isolated by Fredericq (1965), which consists of ColIb–P9 linked to the *try cys* region of *Escherichia coli*. This was transferred to *Salmonella typhimurium* LT2 and a mutant with a de-repressed sex factor isolated by the method of Edwards & Meynell (1968). The mutant plasmid and ColE2 were then introduced into *Salm. typhimurium met A-22 tryB-2* which was mated with its streptomycin-resistant mutant, both Col− and carrying ColIa-CA53. Transfer of the ColI *try*+ plasmid to the ColI+ *try*− recipient was then revealed by the appearance of minute colonies of 'abortive recombinants' resembling those of the abortive transductants described by Ozeki (1956). With both abortive recombinants and abortive transductants a donated genetic fragment fails to replicate normally, but the cause differs in the two systems: in the present experiments an intact replicon is presumably transferred but is prevented from replicating by the superinfection immunity conferred by the recipient's ColIa factor; whereas abortive transductants result from transfer of chromosomal fragments unable to act as replicons.

With the Col− recipient, ColE2 and *try*+ were each transferred at about the same rate: no abortive colonies were seen. With the ColIa recipient, ColE2 was again transferred slightly less often than to the Col− strain. Moreover, minute colonies of abortive recombinants occurred at the same frequency as ColE2, showing that *try*+ and presumably the whole ColI plasmid was being transferred, although it was not otherwise expressed in the majority of recipient colonies.

A minority of stable *try*+ colonies was obtained with both growing and starved I+ recipients but these did not reflect a breakdown in immunity, resulting in the stable co-existence of two independent I factors, because in all of thirty-seven clones examined, the recipient's factor had either disappeared or recombined with the donated plasmid (Edwards & Meynell, in preparation).

The simplest model for abortive gene transfer postulates that the transferred genes fail to replicate in the recipient. Hence, if a colony of an abortive transductant or abortive recombinant is subcultured by spreading, only one colony should be obtained because only one copy of the transferred gene should be present (Stocker, Zinder & Lederberg, 1953; Ozeki, 1956). However, the formation of minute colonies is more complex than the simple model suggests, for, with abortive transductants formed between two histidine-requiring mutants of *Salmonella typhimurium*, spreading may yield many hundreds of minute colonies indistinguishable from the original (Subbaiah & Meynell, 1963). Similarly, spreading colonies of abortive recombinants almost always produced more than one minute colony. Either the transferred genes replicate occasionally or some cells become transiently tryptophan-independent and are able to transmit the wild-type phenotype to a few of their progeny.

Exclusion and superinfection are evidently unrelated phenomena. Exclusion appears to be a widespread characteristic of bacterial conjugation, whether brought about by F or by I. Its cause is unknown but cannot be repulsion of potential donors by sex pili of the recipient. Not only is it rare for even 50 % of F+ cells to form F pili but wild-type I factors like that of ColIa are repressed and fewer than 0·1 % of cells have sex pili (Meynell & Lawn, 1967). Microscopy of strains carrying de-repressed sex factors shows many conjugating cells so that, unless recipient phenocopies are common, exclusion does not result from inhibition of conjugation. Superinfection immunity, on the other hand, is a phenomenon of genetic replication. One can therefore envisage mutant plasmids, analogous to non-excluding phage mutants, which have lost the ability to exclude while still conferring immunity. The alternative, a mutant plasmid retaining exclusion but with a new specificity of replication, might not be able to replicate or its replication might escape its own control and thus be lethal.

SUMMARY

I+ × I+ crosses were studied, using various ColI plasmids. Exclusion due to presence of ColI in recipients was removed by starvation with production of I− phenocopies. Superinfection immunity prevented normal replication of transferred genes: thus, I*trp*+ donated to an I+ *trp*− recipient led to formation of abortive recombinants.

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