Complementation between Filamentous F-specific and I-specific Bacteriophages

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Two groups of filamentous bacterial viruses attacking *Escherichia coli* are known. One of these (Ff; F specific, filamentous, see review by Marvin & Hohn, 1969) adsorbs specifically to the tips of F-pili while the other (If) adsorbs only to the tips of I-pili. The F-pili are produced by cells which possess the F episome or certain derepressed R factors, while the I-pili are produced by cells harbouring Colicine factor I or certain other derepressed R factors (Lawn *et al.* 1967). Several species of Ff are known (fI, fd, M13, ZJ/2, Ec9) but only two isolates of If (If1 and If2). The virus particles of both groups are long flexible rods about 5.5 nm. wide but they differ in length. All the Ff phages are about 850 nm. long whereas the If phages are 1300 nm. long. There is a corresponding difference in the size of the genomes. Both are closed loops of single stranded DNA, but whereas the DNA of Ff phage has a mol. wt of 2 × 10⁶ that of If phage is 3 × 10⁶. The particles of both groups possess antigenic similarities but do not undergo symmetrical neutralization (Meynell & Lawn, 1968).

Studies with amber (am) and temperature sensitive (ts) conditional lethal mutants have revealed eight complementation groups in M13 (Pratt, Tzagoloff & Beaudoin, 1969), six in ZJ/2 (Kay, unpublished), four in fI (Boon & Zinder, 1970) and six in fd (Ohnishi & Kuwano, 1971). No information is available about the genetic constitution of the If phages.

In order to examine the relationship between Ff and If phages more closely an attempt was made to determine whether If phage can complement the am mutants of Ff phage ZJ/2. A suitable non-permissive host organism was prepared by conjugating *Escherichia coli* c3000 F+ (gal+, Sm⁰) with *E. coli* 803 (gal) carrying the resistance factor R64-11 in which the I sex factor is derepressed. By selecting for galactose fermentation and streptomycin resistance a strain, AW2, was isolated which gave plaques with both If 1 and ZJ/2.

Strain AW2 was grown to 5 × 10⁸/ml in Oxoid nutrient broth at 37°. Two ml volumes of culture were infected with either If 1, wild type ZJ/2, one of six amber mutants of ZJ/2 (am 1, 7, 10, 13, 39 and 51, each representative of a different complementation group), or mixtures of each am mutant with If 1, all at a final concentration of 5 × 10⁹/ml. The mixtures were shaken gently at 32° for 10 min. to allow adsorption to occur. After cooling to 4°, washing in ice cold broth and diluting 1:10⁴ in broth the cells were incubated with shaking at 32° for 1 hr. At the beginning and at the end of this period the mixtures were sampled and treated with ether for 1 hr to kill the cells but leave the free phage unharmed. The phage was assayed on both the permissive strain of *E. coli*, cr63 F+ and the non-permissive strain, c3000 F+. The yield of ZJ/2 was 200 p.f.u./cell and the am mutants alone gave about 0.1 p.f.u./cell. In the mixtures all the yields were about 0.1 p.f.u./cell except for am 1 + If 1 in which the yield was 36 p.f.u./cell. There were no counts on the non-permissive host. It is therefore clear that am 1, unlike the other mutants in this collection, can be complemented by If 1 to give a substantial yield of am phage.

Of the ZJ/2 mutants it is known that am 7 corresponds to the M13 gene 2 (RF replication), am 10 to M13 gene 3 (minor coat protein), am 39 to M13 gene 5 (virus strand replication) and am 51 to M13 gene 1 whose function is unknown. None of the six ZJ/2 mutants corresponds to the M13 gene 8 (major coat protein). It is not known to which of the M13 genes the remaining ZJ/2 mutants am 1 and 13 correspond.
Four of the genes of M13 (1, 4, 6 and 7) have unknown functions, but it is unlikely that any of them code for structural components because only two proteins have been found in the virus particle and these have been identified as the products of genes 3 and 8 (Pratt et al. 1969).

By a process of elimination, therefore, it appears that the gene of ZJ/2 whose function is impaired in mutant am I but which can be performed by the product of the corresponding If phage gene is one which may be concerned in some morphopoietic or regulatory activity.

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