Recombination Between Conditional Lethal Mutants Within a Strain of Foot-and-mouth Disease Virus

(Accepted 22 September 1967)

It has been shown previously (Pringle, 1965) that recombination or hybridization can be observed between related but immunologically distinct strains of foot-and-mouth disease virus (FMDV). In order to obtain more precise information concerning the nature of the recombination process in FMDV, suitable single-step mutational changes have been sought which would facilitate intra-strain crosses. Conditional lethal mutants induced by base analogues are described which have been used to demonstrate recombination between mutants considered to affect different viral functions.

Unlike the majority of strains the KENYA-3 strain of FMDV is able to replicate unimpaired at 42° in various host cells. Multiplication of this strain in the presence of the base analogue 5-fluorouracil yields progeny virus with a reduced e.o.p. at 41·5° (Fig. 1). For technical convenience throughout these experiments the e.o.p. was measured at 41·5° rather than 42°, using monolayers in bottles totally immersed in a precision water bath. The decreased e.o.p. at 41·5° is due to the presence of an increasing proportion of clones unable to replicate at elevated temperature. Since it has been shown (unpublished observations) that 5-fluorouracil has no differential selective effect on the multiplication of either the temperature-sensitive (ts) or the normal clones at the non-restrictive incubation temperature (37°), these ts clones are considered to be a product of the mutagenic action of 5-fluorouracil. These observations are similar to those reported earlier for poliovirus by Cooper (1964).

Thirty temperature-sensitive clones, designated ts 1 to 30, were isolated from samples of the KENYA-3 clone replicating in the presence of 100 μg./ml. 5-fluorouracil. No selective procedure was employed in their isolation and the clones were obtained from monolayers possessing a solitary plaque in order to improve cloning efficiency and to provide an accurate estimate of the mutant frequency. The frequency of recovery of ts mutants was about 5·1% at this mutagen concentration, therefore it is probable that 28 or 29 of the 30 ts mutants were single step mutants. The e.o.p. of these clones at 41·5° varied from 10⁻³·5 to 10⁻⁵·5, due mainly to the presence of different proportions of revertants.

Certain pairs of these mutants complemented one another in cells simultaneously infected under restrictive conditions (42°). The yields obtained from individual complementing pairs, however, varied markedly on different occasions and were not simply related to multiplicity. The factors affecting complementation are under investigation.

Recombination was observed consistently, however, and was studied in detail for two mutants, ts 14 and ts 15. These two mutants were assumed to affect different viral function since they complemented one another, and acted at early (ts 14) and late (ts 15) stages of the viral multiplication cycle as determined by temperature shift experiments (unpublished observations).
Fig. 2 illustrates the dependence of recombination on the input multiplicity of the parental mutants under single cycle growth conditions. BHK 21 cell monolayers were infected at multiplicities (p.f.u./cell) between 0.05 and 5. Multiplicity in mixed infection and selfed controls was equated with approximately equal input of each mutant in the mixed infection. A period of 15 min. at 37° was allowed for adsorption, and since FMDV is acid-labile, chilled pH 6.4 phosphate buffer was then added to destroy unadsorbed and unpenetrated parental virus (Cartwright & Thorne, 1958).
Ten min. later the monolayers were washed with chilled medium and then incubated for 5 hr. at the non-restrictive temperature. The cultures were rapidly frozen and later assayed at 37° to give the total titres and at 41.5° to give the titres of non-mutant virus. The yield of non-mutant virus in the self-cross controls (ts 14 + ts 15) increased approximately in direct proportion to multiplicity. In the mixed infection (ts 14 × ts 15) the yield of non-mutant virus rose more steeply. For instance, at a multiplicity of 5 there was a 16-fold excess of non-mutant virus in the mixed infection compared with the summated self-cross controls. This excess represented recombinant virus of the non-mutant type; the reciprocal double mutant recombinant type could not be identified in these experiments. In other experiments recombination could also be detected when the input of the mutant parental virus was not equivalent, provided that one of the mutants was in sufficient excess to infect the majority of the cells.

**Fig. 3.** The time course of appearance of non-mutant virus during a single multiplication cycle. ○ --- ○, selfed control (ts 14 + ts 15) titre at 37°; ● --- ●, mixed infection (ts 14 × ts 15) titre at 37°. ○ --- ○, selfed control (ts 14 + ts 15) titre at 41.5°; ● --- ●, mixed infection (ts 14 × ts 15) titre at 41.5°.

In Fig. 2 it can be see that the relative excess of non-mutant virus in mixed infections is appreciable at multiplicities below unity. This may be an indication that parental viral genomes have been introduced into the host cells which are able to participate in recombination although not themselves competent to initiate productive infections. The high particle/infectivity ratio associated with FMDV (Bachrach, Trautman & Breese, 1964) is consistent with such an assumption.

Fig. 3 illustrates the time course of appearance of non-mutant virus during a single growth cycle. The multiplicity (p.f.u./cell) in this case was approximately 8. The titre of non-mutant virus in the selfed controls (ts 14 + ts 15) paralleled that of the total progeny virus. In the mixed infection (ts 14 × ts 15) the proportion of non-mutant virus increased markedly during the exponential phase of replication, and subsequently remained static, indicating that the excess non-mutant virus was generated during replication (i.e. by recombination) and that it was not accumulated by some undefined selective process. The frequency of the non-mutant class of recombinants in
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this particular experiment was approximately 0.3%, similar to the figure of 0.4% reported by Hirst (1962) and Ledinko (1963) for recombination between mutants of poliovirus resistant to serum inhibitors and guanidine.

I am indebted to W. R. Slade, Mrs Mary O'Sullivan and P. Elworthy for excellent technical assistance, and to Roche Products Ltd., Welwyn Garden City, for a gift of 5-fluorouracil.

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(Received 10 July 1967)