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ASSIGNMENT OF REOVIRUS MUTANT GROUP 'G' TO ITS GENOMIC SEGMENT

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Summary. — Temperature sensitive (ts^+) recombinants between the UV-inactivated reovirus type 1 and the group G ts mutant of reovirus type 3 were obtained using a cross-reactivation technique. Analysis of the dsRNA of recombinant clones in PAGE allowed a preliminary mapping of the ts mutation into its genomic S_3 segment. This was confirmed by the hybridization test.

Key words: reovirus; ts mutant; recombination; ds RNA segments; hybridization ssRNA/dsRNA

Introduction

Several temperature sensitive mutants of type 3 reovirus are known (Cross and Fields, 1977; Hossain and Graham, 1978*a*). Analysis of the recombination frequencies between ts mutants (Fields and Joklik, 1969) showed that the known mutants fall into seven distinct groups, A to G. In the present work, association of the group G ts mutation with its specific dsRNA segment has been approached.

Materials and Methods

Cells and viruses. L cells were grown in suspension or as monolayer cultures in Eagle's minimal essential medium (MEM) supplemented with 5% foetal calf serum. The Dearing strain of reovirus serotype 3 and the Lang strain of reovirus serotype 1 were used. The ts mutant used was R_2G (453). Each strain of virus was plaque-purified, grown up into a large lysate and the harvested virus was then purified for subsequent use. The virus was assayed by standard plaque method or by an infectious center counting procedure as described (Hossain and Graham, 1978*a*, *b*). The permissive temperature for the ts mutant assay was 31 °C and the non-permissive one 39 °C.

UV-inactivation. Purified virus sample in phosphate buffered saline (PBS) was sonicated for three times 30 sec intervals in ice bath to break up clumps, and then UV-inactivated as described (Hossain and Graham, 1978*a*, Hossain, 1981b).

Preparation of ^{32}P-labelled dsRNA from the recombinant viral clones. Clones of suspected viral recombinants were obtained by a cross-reactivation technique described in Results. To examine the nature of the genomic segmens of these clones, the viral dsRNA was first labelled with ^{32}P. Lysates obtained by serial passage of cloned virus in L cells, with titres of approximately 10^8 PFU/ml, were used to infect confluent monolayer of L cells. Three plates 10 cm in diameter were infected with each clonal isolate at a multiplicity of infection (MOI) of 5—15 PFU/cell

Table 1. Cross reactivation of type 3 ts mutant (G) with UV-irradiated type 1 reovirus

Virus	MOI	Infectious centres (%)
Type 1 (UV-irradiated)	0.2	2.3*
Type 3 ts G (453)	3	1.6
UV-type 1 + type 3 G	0.2 + 3	13.4

* Percentage of plated cells giving infectious centres after incubation at 31 °C for 40 hr and shifting up to 39 °C.

After absorption for one hr, 15 ml of MEM containing 2% foetal calf serum was added to each plate at 31 °C. Four hr later the medium was replaced by a medium containing phosphate-free MEM, 1% foetal calf serum (dialysed against Earles' salt solution), 0.5 μg/ml of actinomycin D and 88.8 MBq of ^{32}P per plate. After 46—48 hr at 31 °C when approximately one-third of the cells had been lysed, the monolayers were scraped with a rubber policeman and cells were centrifuged and washed with PBS. The cell pellet was resuspended in 2 ml of LTM buffer (0.14 mol/l LiCl; 0.1 mol/l Tris-chloride, pH 7.4; 0.001 mol/l $MgCl_2$). SDS (sodium dodecyl sulphate) was added to 0.5% concentration and the mixture extracted with phenol. Yeast tRNA (50 mg/ml) was added to the aqueous phenol-extracted solution to act as a carrier followed by 3 vol of ethanol and the mixture was placed to −20 °C for 18 hr. The resulting precipitate was centrifuged, dissoloved in 0.2 ml of LTM buffer and DNase (RNase-free) was added to a concentration of 10 μg/ml for 30 min at 37 °C. The ssRNA was then precipitated by addition of LiCl to a concentration of 1 mol/l, the mixture was kept at 4 °C for 18 hr and centrifuged. The supernatant solution, containing the ^{32}P-dsRNA, was mixed with 3 volumes of ethanol and after standing for 18 hr at −20 °C the resulting precipitate of dsRNA was centrifuged, dissoloved in 0.01 mol/l STE (0.01 mol/l NaCl in 0,05 mol/l Tris-chloride, pH 7.4; 0.001 mol/l EDTA) and analysed by polyacrylamide gel electrophoresis (PAGE) as previously reported (Hossain and Graham, 1978*a*).

Preparation of ^{3}H-labelled ssRNA transcripts from the recombinant viral clones. Lysates were prepared from suspected recombinant viral clones as described in the previous section and used to infect 100 ml suspension cultures of L cells (5×10^8 cells/ml) at MOI of 5—15 PFU/cell by 31 °C. ^{3}H-labelled ssRNA transcripts were prepared from these cells by a method described elsewhere (Hossain and Graham, 1978*a*); the ^{3}H-label has been introduced into the ^{3}H-ssRNA utilizing ^{3}H-uridine labelled at 31 °C; the ssRNA was subsequently used for the hybridization procedure as described in next section.

Hybridization of ^{3}H-labelled ssRNA transcripts from recombinant genomes with ^{14}C-labelled dsRNA. Hybridization was performed by mixing ^{3}H-labelled ssRNA transcripts with excess of ^{14}C-labelled dsRNA obtained from puribed wild-type reovirus. The dsRNA had been denatured with 9 volumes of DMSO (dimethyl sulphoxide) at 37 °C for 1 hr. The resulting hybrids dissolved in 0.3 mol/l STE (0.3 mol/l NaCl; 0.05 mol/l Tris-chloride, pH 7.4; 0.001 mol/l EDTA) were mixed with T_1 RNase to a concentration of 10 μg/ml and left for 30 min at 37 °C. Then the RNase was removed, 3 volume of ethanol were added to the aqueous extract and after 18 hr at −20 °C the precipitated, hybrids were centrifuged, dissolved in 0.01 mol/l STE buffer and analysed by polyacrylamide gel dectrophoresis (PAGE).

PAGE of hybridized ssRNA/dsRNA segments. Analyses were carried out in 5% polyacrylamide gel slabs as reported earlier (Hossain and Graham, 1978*a*). For autoradiography, the gels were dried and exposed on Kodak X-Omat blm. To measure both ^{3}H- and ^{14}C-activities, the gels were cut into 1 mm slices, solubilized in 50% H_2O_2 and the radioactivity was determined by sci ntillation counting.

Results

Preliminary mapping of the group G mutation

A cross reactivation experiment between the G (453) mutant (MOI = 3 PFU/cell) ad the UV-irradiated type 1 virus (approximate survival 10^{-2}, MOI = 0.2 PFU/cell) was carried out. The infectious centres were first

plated at 31 °C for 40 hr to permit recombination and then raised to 39 °C to allow ts$^+$ plaques to form. Under these conditions plaques could arise at 39 °C in several ways: from multiplicity reactivated type 1 virus; from ts$^+$ revertant of the G mutant; through leakiness of the mutant; or from ts$^+$ recombinant in which the type 3 genomic segment containing the ts mutation

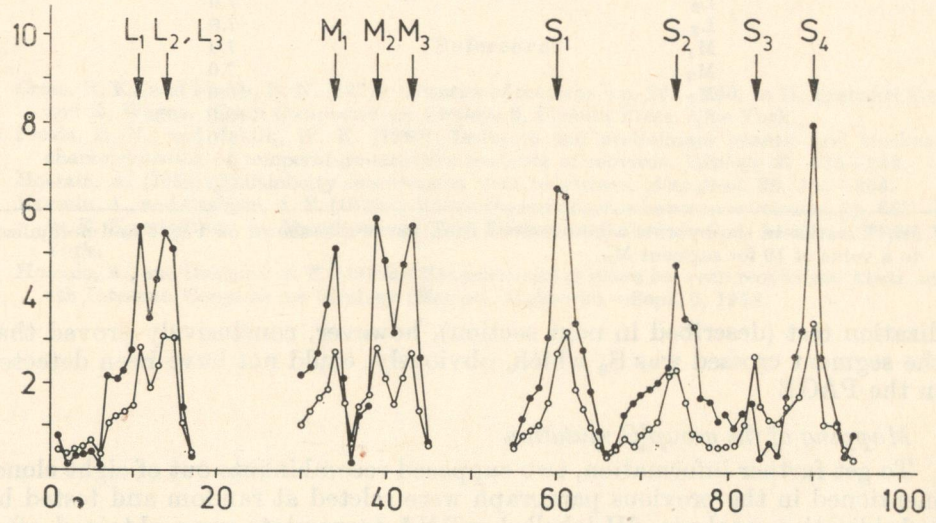

Fig. 2.
PAGE analysis of the reovirus hybrids
Hybrids were formed between ^{14}C-labelled dsRNA of type 3 reovirus and ^{3}H-labelled transctipts from a recombinant clone selected from the cross of UV-irradiated type 1 and the ts G (453) mutant of type 3. The hybrids were digested with T_1 RNase before analysis as described in Materials and Methods.
^{3}H-label (●———●-, ^{14}C-label (○———○)
Abscissa: fraction number; ordinate: for — ^{3}H cpm × 10^{-3}; for — ^{14}C cpm × 10^{-2}.

was replaced by the equivalent unhit type 1 segment. The results of the cross reactivation experiment arre shown in Table 1. Examination of the last column indicates that with this mutant tested in the coinfection with the irradiated type 1 virus, approximately two out of three plaques originated from the ts$^+$ recombinant.

A number of ts$^+$ clones obtained by the cross reactivation experiment were subjected to further analysis. The dsRNAs from eight clones were labelled with ^{32}P and analysed in PAGE. A representative set of such analyses shown in Fig. 1 indicates that the genomes of all the suspected recombinants are of type 3. Under the conditions of PAGE analysis, only certain segments like M_1, M_2, S_1 and S_4 can be differentiated between the type 1 and type 3 reoviruses. The clones selected from the crosses were proved to represent actual recombinants, but if any recombinants had been formed in the cross with the reassorted type 1 viral segment remained undetectable. The hybri-

Table 2. Ratios of $^{3}H/^{14}C$ in hybrids formed between ^{14}C-labelled dsRNA of type 3 virus and ^{3}H-labelled transcripts from a recombinant between UV-irradiated type 1 reovirus and the ts G (453) type 3 mutant

Genome segment	$^{3}H/^{14}C$ ratio*
L_1	8.0
L_2	7.0
L_3	7.0
M_1	7.0
M_2	7.0
M_3	10.0
S_1	12.0
S_2	8.0
S_3	0
S_4	9.0

* $^{3}H/^{14}C$ ratios in the hybrids were obtained from the profile shown on Fig. 2 and normalized to a value of 10 for segment M_3.

dization test (described in next section), however, conclusively proved that the segment crossed was S_3 which, obviously, could not have been detected in the PAGE.

Mapping of the group G mutation

To get further information, two supposed recombinants out of eight clones mentioned in the previous paragraph were selcted at random and tested by hybridization analysis. ^{3}H-labelled ssRNA transcripts were obtained after infection of L cells by the 'recombinant' virus and hybridized to ^{14}C-labelled dsRNA from type 3 virus. The hybrids were digested by T_1 RNase and analyzed by PAGE. Fig. 2 shows the pattern obtained for one of the clones, the second clone gave a similar picture. The $^{3}H/^{14}C$ ratios for the peaks are shown in Table 2; it is apparent that the S_3 peak contains relatively little or no ^{3}H-label. This indicates the absence of the type 3 S_3 dsRNA segment in the G mutant and in the recombinant; further it confirms that the S_3 segment was indeed exchanged from the type 1 because of the lack of homology done prior to analysis of the hybrids in PAGE. The controls did include ^{3}H-ssRNA (reo 3) hybridized to ^{14}C-dsRNA (reo 3) done simultaneously. Therefore, the group G ts mutation of type 3 virus has been assigned to the S_3 segment of the genome.

Discussion

A number of ts mutants of type 3 are known (Cross and Fields, 1977; Hossain and Graham, 1978*a*). The group E ts mutant and the group F ts mutant of reovirus type 3 have been already mapped into their genomic segments (Hossain and Graham, 1978*a*). This paper has provided the assignment of the group G ts mutant into its genomic segment. The efficient method of cross reactivation devised earlier has been successfully utilized here to obtained recombinants between the reovirus serotypes. The re-

combinant clones obtained by the cross of type 3 ts mutant G with UV-inactivated type 1 have been analyzed by PAGE. In this case, however, the absence of a detectable type 1 virus segment in the clones had made the mapping of the ts G mutation difficult. Nevertheless, a conclusive result had been obtained by the hybridization test, whereby the G mutation has been shown to be associated with the S_3 segment.

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