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SEQUENCE HOMOLOGY BETWEEN HUMAN REOVIRUSES

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Summary. — The extent of homology between the human reoviruses type 1, 2 and 3 have been studied by hybridization technique. The results suggest that the three types share substantial homologies in the large (L) segments and very little in the medium (M) and small (S) segments.

Key words: *reovirus RNA segments*; *sequence homology*; *RNA/RNA hybridization*

Introduction

The human reoviruses, of which there are three serologic types, are distinguished from other viruses by two main features: (1) the genomes are comprised of double-stranded (ds) RNA (Gomatos and Tamm, 1963; Watanabe and Graham, 1967); (2) the genomes consist of discrete and unique dsRNA segments (Bellamy *et al.*, 1967; Watanabe and Graham, 1967). Ten characteristic segments are distinguishable by polyacrylamide gel electrophoresis (PAGE); furthermore, differences had been detected in the mobilities of some identical segments coming from different serotypes (Hossain and Graham, 1978*a*, *b*; Hossain, 1982*a*, *b*, *c*).

It has been shown earlier (Martinson and Lewandowski, 1974), that the sequences of reovirus type 2 and 3 appear to have little in common and that those of serotypes 1 and 3 share imperfect homologies. These authors had used RNA/RNA reannealing studies in conjunction with the analysis of the reannealed RNA duplexes by hydroxylapatite chromatography. In present paper, the question of homology between the reovirus serotypes has been investigated by using an entirely different highly sensitive technique, namely the hybridization test and PAGE analysis (Hossain and Graham, 1978*a*, *b*).

Materials and Methods

Cells and virus. L cells were grown in suspension or as monolayer cultures in Eagle's minimal essential (MEM) medium supplemented with 5% foetal calf serum. The Dearing strain of reovirus serotype 3, the Lang strain of reovirus serotype 1 and the Jones strain of type 2 virus were plaque-pufiried, grown up into a large lysate and then the virus was purified for various experiments.

Preparation of ^{14}C-labelled dsRNA from the serotypes. Suspension cultures of L cells were centrifuged at 600 × g for 10 min and the cells were suspended in MEM at a concentration of 10^7 per ml. Purified virus was added at mutiplicity of infection (MOI) of 20 PFU/cell. After 2 hr adsorption at continuous stirring with a magnetic bar, the cells were centrifuged and suspended to a concentration of 5×10^5 per ml in MEM containing 2% foetal calf serum (FCS). Magnetically stirred infected suspension cultures were incubated at 31 °C, the cells were harvested approximately after 40 hr, centrifuged at 600 × g for 20 min and the sediment was resuspended in TNM buffer (0.025 mol/l NaCl, 0.001 M Tris-HCl, pH 7.4, 0.011 mol/l mercaptoethanol). This suspension was homogenized with Freon 113 and sedimented onto a CsCl cushion. The virus band was collected, dialyzed against SSC (0.015 mol/l sodium citrate, pH 7.4) and further sedimented at 27,000 rev/min for 1 hr in a SW 27.1 Beckman rotor. The pellet was suspended after sonic treatment in 2 ml of SSC buffer and the suspension layered on the top of 20 to 40% sucrose gradient (centrifuged in SV/27 rotor for 90 min at 25,000 rev/min). The virus band formed was collected, dialyzed against SSC overnight and further centrifuged throught a second CsCl gradient (1.32 to 1.43 g/ml) for 2 hr at 25,000 rev/min in the SW27 rotor. The virus band was isolated and dialyzed against SSC. For labelling, actinomyxin D (0.5 μg/ml) was added to the culture at zero time of infection along with 0.74 kBq of ^{14}C-uridine per ml (1.85 GBq/ml.

Preparation of ^{3}H-labelled single-stranded (ss) RNA transcripts from each serotype. Lysates were prepared from the purified infectious reovirus stocks of all three serotypes; 100 ml suspension

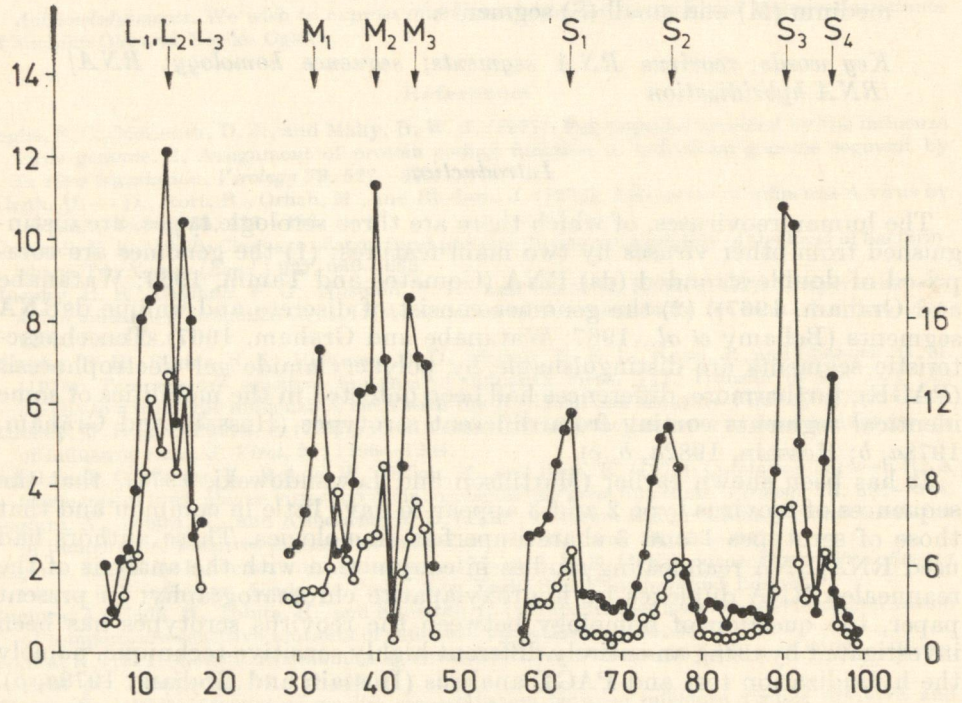

Fig. 2.

PAGE analysis of the hybrids formed between type 3 ^{14}C-dsRNA and type 3 ^{3}H-labelled transcripts The hybrids were digested with T_1 RNase before analysis as described in Materials and Methods. Normal run of the gel for 42 hr. ^{3}H-label (●——●), ^{14}C-label (○——○).
Abscissa: fraction number; left ordinate: ^{3}H-counts/min × 10^{-3}; right ordinate: ^{14}C-counts/min × 10^{-2}.

cultures of L cells in concentration of 5×10^6 cells/ml were inoculated at MOI of 10 PFU/cell and incubated at 31 °C. Actinomycin D was added in a concentration of 0.5 μg/ml 4 hr after infection along with 92.5 MBq of ^{3}H-uridine. By 20 hr post infection the cells were centrifuged, washed with PBS and resuspended in 4 ml of LAM buffer (0.14 mol/l LiCl, 0.01 mol/l sodium acetate; 0.001 mol/l $MgCl_2$; pH 5.5). Sodium dodecyl sulphate (SDS) was added to 0.5% concentration and the solution was extracted with phenol. To the resulting aqueous solution LiCl was added to reach 1 M concentration and the mixture placed to 4 °C for 18 hr. The resulting precipitate of ^{3}H-ssRNA was centrifuged, redissolved in LAM buffer and again precipitated by addition of LiCl to 1 M concentration. Finally, the precipitate was centrifuged, dissolved in 0.03 M STE buffer (0.03 mol/l NaCl; 0.05 mol/l Tris-HCl, pH 7.4, 0.001 mol/l EDTA) and used in the hybridization procedure as decribed in the next section.

Hybridization of ^{3}H-labelled ssRNA transcripts with ^{14}C-labelled genomic dsRNA. Hybridization was performed by mixing the ^{3}H-ssRNA transcripts with excess of ^{14}C-dsRNA obtained from the purified virus. The resulting hybrids in 0.3 mol/l STE buffer (0.3 mol/l NaCl; 0.05 mol/l Ttis-HCl, pH 7.4; 0.001 mol/l EDTA) were mixed with T_1 RNAase to a concentration of 10 μg/ml and left for 30 min at 37 °C; the RNase was then removed by phenol extraction. Three volumes of ethanol were added to the quesous extract and after 18 hr at —20 °C the precipitated hybrids were centrifuged, dissolved in 0.01 mol/l STE buffer and analyzed by PAGE.

Polyacrylamide gel electrophoresis. Analyses were carried out on 5% polyacrylamide gel slabs (20 cm long, 15 cm wide and 0.15 cm thick). Gells were formed with 5% acrylamide, 0.125% N,

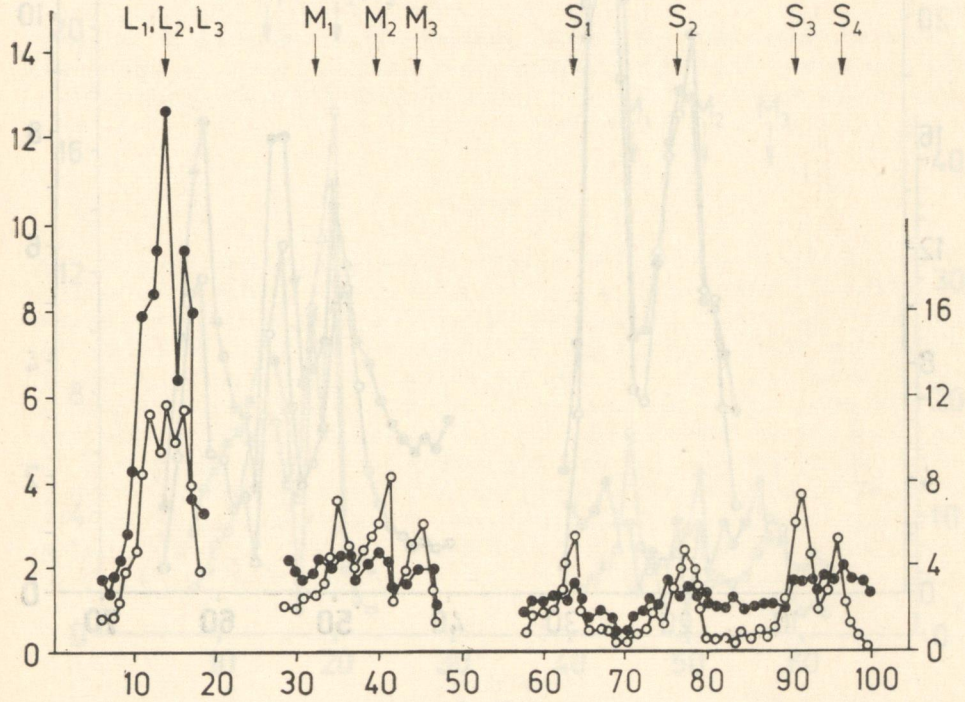

Fig. 3.

PAGE analysis of the hybrids formed between type 3 ^{14}C-labelled dsRNA and type 1 ^{3}H-labelled transcripts

Abscissa, ordinates and symbols as in Fig. 2.

N-methylene-bisacrylamide in 0.04 mol/l Tris-HCl; 0.001 mol/l EDTA; pH 7.4; 0.04% TEMED and the polymerization was catalyzed by 0.5% ammonium persulphate. Gels were run at a constant current of 45 mA for 42—46 hr with at least two changes of electrophoresis buffer (0.04 mol/l Tris-HCl; 0.05 mol/l sodium acetate; 0.001 mol/l EDTA; 0.05 mol/l sodium acetate; 0.001 mol/l EDTA; pH 7.4) in the end trays. For autoradiography the gels were dried and exposed on Kodak X-Omat film. To measure ^{3}H and ^{14}C counts, the gels were cut into 1 mm slices; the slices were solubilized in 50 % H_2O_2 and the radioactivity was determined by scintillation counting in a Beckman LS-250 liquid scintillation spectrometer.

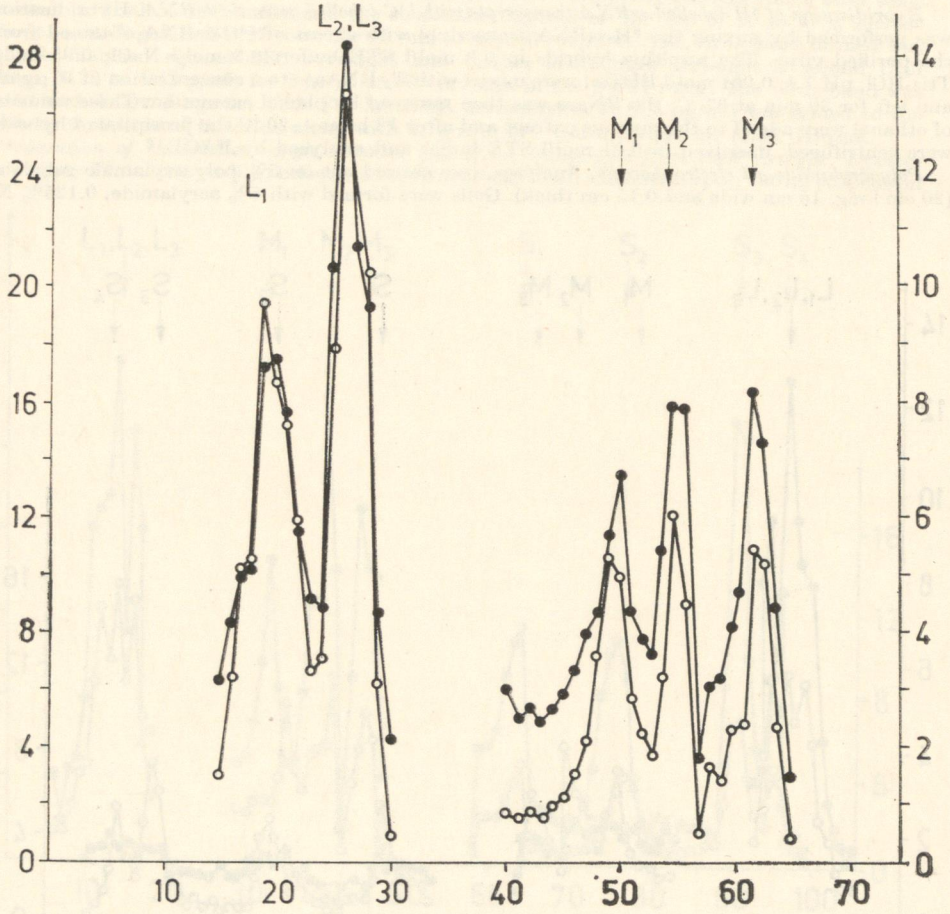

Fig. 4.

PAGE analysis of the hybrids formed between type 3 ^{14}C-labelled dsRNA and ^{3}H-labelled transcripts from the homologous type 3

Abscissa, ordinates and symbols as in Fig. 2.

Results

Analysis of the genomes in PAGE

^{14}C-dsRNA of the reovirus serotypes 1, 2 and 3 was prepared and analyzed in PAGE (Fig. 1, Plate V). Ten discrete segments were seen in each case. The mobilities of segments M_1, M_2, S_1, S_2, S_3, S_4 of the three serotypes appeared to be characteristically different.

Homology between the reovirus serotypes

^{3}H-ssRNA transcripts were first hybridized to homologous ^{14}C-dsRNA; the hybrids formed were digested with T_1RNase and analyzed by PAGE. A representative set of such analysis is shown in Fig. 2 showing the results of the annealing of ^{3}H-labelled type 3 ssRNA to type 3 ^{14}C-dsRNA. This was the control for comparison with the heterologous hybridization.

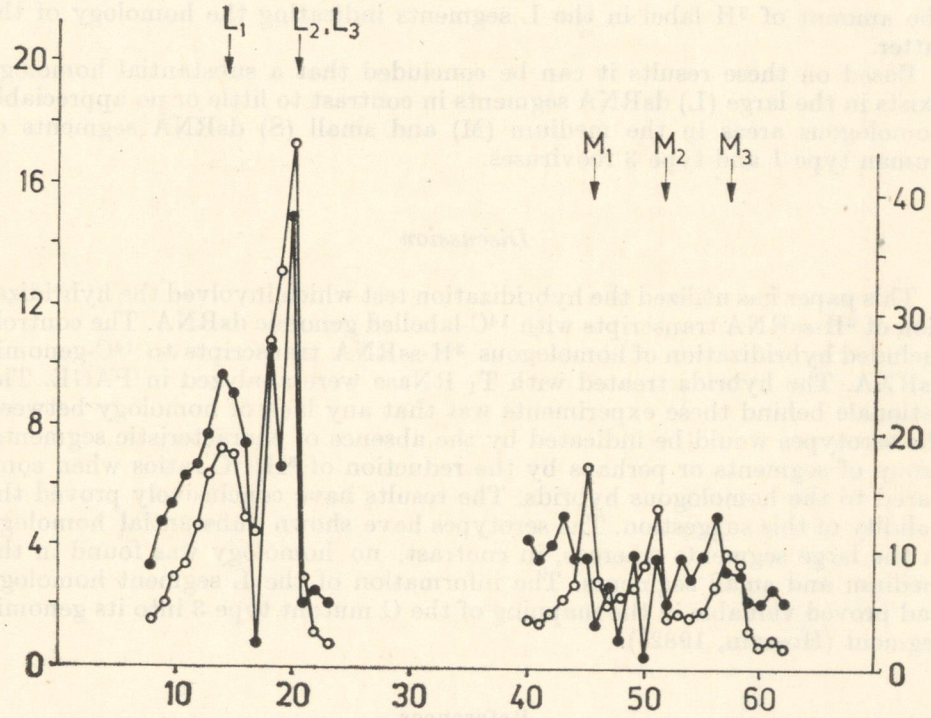

Fig. 5.

PAGE analysis of the hybrids formed between type 3 ^{14}C-labelled dsRNA and type 1 ^{3}H-labelled transcripts

Abscissa, ordinates and symbols as in Fig. 2.

Fig. 3 shows the results of heterologous hybridization of type 3 ^{14}C-dsRNA with type 1 ^{3}H-ssRNA. The hybrids were digested with T_1RNase to remove the non-homologous areas. A substantially significant reduction or absence of ^{3}H peaks corresponding to the M and S segments could be found in contrast to very little change in ^{3}H/^{14}C ratio in the L segments. This indicates a total lack of homology between the M and S segments of the human type 1 and 3 reoviruses and suggests the appreciable homology of the L segments.

In the effort to further elucidate the homology of the L segments between the reovirus serotypes 1 and 3, PAGE analyses were carried out for an extended period of time so that the S segments migrated off the gel to achieve a better separation of the L segments. Fig. 4 shows the results of the homologous hybridization between type 3 ^{14}C-dsRNA with type 3 ^{3}H-ssRNA run in PAGE for an extended period of time. The purpose was the control of the heterologous hybridization of type 3 ^{14}C-dsRNA with type 1 ^{3}H-ssRNA. The hybridization product was subjected to T_1 RNase digestion and analyzed by PAGE for an extended period of time. It can be seen from Fig. 5 that there was a loss or reduction of ^{3}H label in the M segments as compared to the amount of ^{3}H label in the L segments indicating the homology of the latter.

Based on these results it can be concluded that a substantial homology exists in the large (L) dsRNA segments in contrast to little or no appreciable homologous areas in the medium (M) and small (S) dsRNA segments of human type 1 and type 3 reoviruses.

Discussion

This paper has utilized the hybridization test which involved the hybridization of ^{3}H-ssRNA transcripts with ^{14}C-labelled genomic dsRNA. The controls included hybridization of homologous ^{3}H-ssRNA transcripts to ^{14}C-genomic dsRNA. The hybrids treated with T_1 RNase were analyzed in PAGE. The rationale behind these experiments was that any lack of homology between the serotypes would be indicated by the absence of characteristic segments, group of segments or perhaps by the reduction of ^{3}H/^{14}C ratios when compared to the homologous hybrids. The results have conclusively proved the validity of this suggestion. The serotypes have shown substantial homology in the large segments whereas, in contrast, no homology was found in the medium and small segments. The information of the L segment homology had proved valuable in the mapping of the G mutant type 3 into its genomic segment (Hossain, 1982*b*).

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