THE GENOME OF TRANSMISSIBLE GASTROENTERITIS VIRUS (TGEV)

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Lysis of isolated TGE virions with sodium dodecyl sulphate (SDS) at room temperature followed by electrophoresis, yields predominantly a single band of RNA running at about molecular weight 6.8 x 10^6. Heating the SDS lysate at 80°C for 15 min prior to electrophoresis results in a cluster of bands of between 0.2-6.8 x 10^6. Electrophoresis of nucleic acids isolated from the SDS lysate using phenol, at room temperature, also gave several discrete bands some of which co-migrated with TGEV subgenomic mRNA species isolated from infected cells.

Single gene lengths of TGEV RNA would be valuable in recombinant nuclei acid technology aimed at antigen production in vitro. Thus experiments were designed to determine whether the instability of TGEV genomic RNA could be manipulated to produce single gene lengths. The factors controlling the break-down of TGEV genomic RNA into discrete fragments were sought by varying virus growth and purification conditions, and also varying the methods used for isolating RNA from virions. Of all the studies where growth and virion purification conditions were varied, only increasing the harvest time from 15 h to 18 h had significant effect showing an increased degradation of genomic RNA. However this was not into the discrete bands observed earlier, but rather into smaller pieces of less than 3 x 10^5. Varying the methods of isolating virion RNA all had some effect on instability. In general the greater the chaotropic potential of the method the greater the degree of break-down into discrete RNA species (see photograph). Adding nuclease inhibitors to virions before phenol extraction of the RNA did not decrease the degree of break-down. Similar break-down was observed when genomic RNA was extracted from a low melting point gel and re-electrophoresed.

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The instability of TGEV genomic RNA appears to be inherent in its structure, and break-down of the RNA into specific bands can be manipulated to some extent by controlling the conditions of nucleic acid purification. The possibilities of using this manipulation to produce pieces of RNA for cloning are being explored. The advantages of this approach over isolating TGEV subgenomic mRNA species from infected cells are:

1. Contamination with host cell RNA should be minimal

2. The subgenomic mRNAs consist of a nested set having common 3' end sequences. It is possible that if break-down of TGEV genomic RNA is a reflection of its structure, some of the pieces produced may contain the messages required without the common 3' ends which may be undesirable for <u>in vitro</u> expression via cloning techniques.

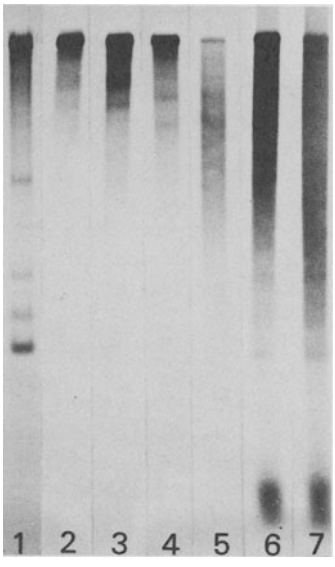

Figure 1

Channel 1) sub-genomic messenger preparation from infected cells
" 2) SDS lysis at room temp
" 3) SDS lysis + proteinase K at room temp
" 4) SDS lysis + proteinase K at 60°C
" 5) SDS lysis + proteinase K at 80°C
" 6) SDS lysis ⟶ 1 M sodium perchlorate ⟶ chloroform/ isoamyl alcohol
" 7) SDS lysis ⟶ phenol