Influence of the Host Cell on the Genomic and Subgenomic RNA Content of Defective-interfering Influenza Virus

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SUMMARY

Clonally derived stocks of defective-interfering (DI) influenza virus prepared in chick embryo fibroblast (CEF) cells were all deficient in genomic RNAs 1, 2 and 3 but had different patterns of subgenomic RNAs. A single passage at high multiplicity in L cells altered the pattern of genomic RNAs independently of the subgenomic species, while in BHK cells the opposite situation prevailed. Therefore, there is no simple relationship in DI influenza virus between the loss of genomic RNA segments and the presence of subgenomic RNAs.

The host cell plays an important role in the generation and amplification of a number of defective-interfering (DI) viruses and this is particularly well documented for vesicular stomatitis virus (for review, see Reichman & Schnitzlein, 1979). The generation of DI Semliki Forest virus is also strongly influenced by the host cell (Stark & Kennedy, 1978) and the production of non-infectious influenza virus is similarly affected (Choppin, 1969). However, analysis of viral RNA in the latter study (Choppin & Pons, 1970) was incomplete since suitable techniques were not then available.

Attention was focused on the distribution of RNA in DI influenza virus when we showed that, as the p.f.u. : HA ratio fell with successive infections at high multiplicity, genomic RNA segments 1, 2 and 3 also decreased and a number of subgenomic RNAs became plainly apparent (Crumpton et al., 1978). However, analysis by oligonucleotide mapping showed that the same subgenomic RNAs were present in smaller amounts in standard virus (Crumpton et al., 1979). These studies brought together early work which had shown that the RNA content of 'von Magnus' virus was less than that of infectious virus (Ada & Perry, 1955; Choppin & Pons, 1970; Duesberg, 1968; Nayak, 1972) and that 'small' RNAs (Hay et al., 1977; Palese & Schulman, 1976) were present in DI virus. The association of subgenomic RNAs specifically with DI virus and the loss of genomic RNAs has been found in other systems (Nakajima et al., 1979; Nayak et al., 1978; Janda et al., 1979). Oligonucleotide mapping confirmed that subgenomic RNAs were entirely viral in origin (Crumpton et al., 1979; Davis & Nayak, 1979). The nature of interference by DI influenza virus is by no means clear. In all other virus systems DI virus solely contains DI RNA, a short version of the genome. However, in DI influenza virus stocks there are not only several subgenomic RNAs (putative DI RNA; Nayak, 1980) but also the majority of genomic RNA segments. Although Janda & Nayak (1979) have shown that subgenomic RNPs can cause interference in vivo, their mode of action and the significance of the presence of genomic RNAs in DI particles remain to be explored.

In this report we have investigated the role of the host cell in determining the distribution of genomic and subgenomic RNAs present in DI influenza virus. We used the Dobson variant of A/FPV/Dutch/27 (HavlNeq1) (Zavada, 1969) which was grown in chick embryo fibroblast (CEF), hamster (BHK-21F) or mouse (L929) cells (Crumpton et al., 1978).

Virus was cloned by three sequential plaque isolations in CEF cell monolayers and stocks were grown in embryonated eggs (Table 1). Confluent CEF monolayers in rolled Winchester bottles were infected with 4 ml inoculum for 30 min at 37 °C. The cell sheets were then washed twice with saline (0-15 M-NaCl), 50 ml TCF (Glasgow minimal essential medium
Table 1. Production of standard and DI virus in CEF cells using virus cloned in CEF cells as inoculum

<table>
<thead>
<tr>
<th>Clone</th>
<th>M.o.i.*</th>
<th>Inoculum (log_{10} p.f.u.:HA)</th>
<th>Progeny (log_{10} p.f.u.:HA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>194</td>
<td>0.1†</td>
<td>5.6</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>10.0†</td>
<td>5.6</td>
<td>2.2</td>
</tr>
<tr>
<td>0.6‡</td>
<td>4.4</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>0.09†</td>
<td>5.8</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>9.0‡</td>
<td>5.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>0.17†</td>
<td>4.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>0.004†</td>
<td>5.5</td>
<td>4.3</td>
</tr>
<tr>
<td>0.4‡</td>
<td>5.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>0.03‡</td>
<td>3.3</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

* Approx. 1.5 × 10⁶ cells were inoculated.
† Inoculum (infectious allantoic fluid) produced by infecting eggs with about 10⁴ p.f.u.
‡ Inoculum (infectious allantoic fluid) produced by inoculating eggs with about 10⁸ p.f.u. (i.e. one undiluted passage of allantoic fluid).

plus 0.5% newborn calf serum; Flow Laboratories) were added and incubation was continued for 1 h at 37°C. Virus that had eluted was then removed with a further saline wash, 80 ml TCF were added and the cells were incubated at 37°C for 24 h. ³²P-labelled virus was prepared in the same way and virus multiplication was determined by assaying for haemagglutination (HA) and infectivity, all as previously described (Crumpton et al., 1978). Table 1 shows the p.f.u.:HA ratios of DI virus progeny arising from three of the virus clones. Each formed DI virus with varying facility. For example, clone 211 produced DI virus so readily that the original seed had to be diluted 10-fold more than the other two clones in order to produce progeny with a p.f.u.:HA ratio > 10⁴. RNAs of standard and DI viruses derived from four clones were analysed by polyacrylamide gel electrophoresis (PAGE), as previously described (Crumpton et al., 1978). Genomic RNAs of standard virus preparations from each clone were identical (e.g. Fig. 1 A, track e). DI virus stocks prepared from all clones lost RNAs 1, 2 and 3 relative to RNAs 4 to 8 (Fig. 1 A, tracks a to d). In contrast, there were marked differences in the pattern of subgenomic RNAs of the different DI viruses. Multiple subgenomic RNAs were always seen, but were weakly labelled (and hence often difficult to reproduce photographically). The arrowheads in Fig. 1 (A) indicate the subgenomic RNAs which were reproducibly found over a number of experiments. The six subgenomic RNAs which we previously reported for clone 194 (Crumpton et al., 1978) can now, under favourable conditions, be resolved into eight discrete segments. The distribution of subgenomic RNAs of clones 194 and 210 was very similar in migration and proportion (Fig. 1 A, tracks c and e), while those of clones 211 and 212 differed in mobility from 194 and each other (compare Fig. 1 A, tracks a and b, c and d). It should be noted that the same patterns of subgenomic RNAs were always seen in standard virus preparations as in resulting DI virus, although the proportion of subgenomic to genomic RNAs was less (see Fig. 1 A, track e; 1 B, tracks a and c; and W. M. Crumpton et al., unpublished data). Janda et al. (1979) made similar observations of variation in subgenomic RNAs of a number of WSN clones prepared in MDBK cells. They also found a variable loss of genomic RNAs 1, 2 or 3 and a variable gain in RNA 7, whereas in our experience RNAs 1 to 3 only decreased. However, the samples are too small to know whether such differences are significant.

We investigated the effect of the host cell in controlling the genomic and subgenomic profiles of RNA of DI virus by making a single passage in BHK or L cells with clone 194 as described in the legend to Fig. 1 (B). DI virus prepared in both cell types showed reduction of RNAs 1, 2 and 3 but L cell-grown DI virus was also deficient in RNA 6 and, to a lesser
Fig. 1. PAGE of RNA extracted from DI and standard FPV purified from tissue culture fluids. (A) Different clones grown in $^{32}$P-labelled CEF cells. The inoculum was standard virus passed once undiluted through embryonated hen's eggs as described in Table 1. (a) DI virus clone 194 (p.f.u.: HA = $10^{2.1}$); (b) DI virus clone 212 (p.f.u.: HA = $10^{1.4}$); (c) DI virus clone 210 (p.f.u.: HA = $10^{2.4}$); (d) DI virus clone 211 (p.f.u.: HA = $10^{1.6}$); (e) standard virus clone 194 (p.f.u.: HA = $10^{4.9}$). (B) Clone 194 grown in $^{32}$P-labelled BHK, L and CEF cells. The inoculum for BHK cells was standard virus passed once undiluted through hen's eggs and for L cells was standard virus. (a) CEF standard virus (p.f.u.: HA = $10^{4.6}$); (b) BHK DI virus (p.f.u.: HA = $10^{3.7}$); (c) CEF standard virus (p.f.u.: HA = $10^{6.4}$); (d) L cell DI virus (p.f.u.: HA = $10^{7.8}$).
extent RNA 5 (Fig. 1B, track d). BHK-grown DI virus had about 14 subgenomic RNAs of which at least six were new. The others had approximately the same mobilities as those produced in CEF cells (Fig. 1B, track b). In L cell-grown DI virus the pattern of subgenomic RNA was unchanged from that of the CEF parent, even though the L cell DI virus was deficient in genomic RNA segments 5 and 6 as well as 1, 2 and 3. A number of points emerge from the above analysis.

First, the data show that virus grown in chicken cells had a characteristic pattern of subgenomic RNAs which was determined by a particular virus clone. On high multiplicity passage in CEF cells, all clones lost RNAs 1 to 3 and showed an increase in the proportion of subgenomic RNAs (relative to genomic RNAs), but their distribution remained unaltered. High multiplicity passage of CEF-grown standard virus in different host cells either changed the pattern of genomic RNA without altering the subgenomic (L cells) or the subgenomic without affecting the genomic (BHK cells). Clearly, the loss of genomic RNA segments is not necessarily reflected in the appearance of subgenomic species and vice versa.

Second, DI viruses lose not only RNA segments 1, 2 and 3 (Crumpton et al., 1978; Janda et al., 1979) but also other segments such as 5 and 6 in L cells and RNA 5 in virus adapted by low m.o.i. passage to BHK cells (W. M. Crumpton et al., unpublished data). Thus, although there is a preferential loss of RNAs 1 to 3, others are also susceptible. At this stage it is not clear how the action of DI virus results in the loss of only certain RNA segments and why this loss should be cell-type specified.

Third, even the most infectious virus (p.f.u.:HA = 10^4) contained subgenomic RNAs. These increased in DI virus preparations but, for a 40000-fold reduction in p.f.u.:HA, the subgenomic RNAs increased by only 2.6-fold (W. M. Crumpton et al., unpublished data). What then is the case for subgenomic RNAs being DI RNAs (Nayak, 1980)? There is the circumstantial evidence that infectious WSN can be obtained without detectable subgenomic RNAs and that in some DI virus stocks there is a massive increase in one or more subgenomic RNAs such that genomic and subgenomic segments are labelled with equal intensity (Nayak et al., 1978; Nakajima et al., 1979). Clearly, this situation does not include our system and cannot therefore provide an all embracing explanation. On the other hand, the case is supported by the small u.v. target size of interference relative to infectivity (Nayak et al., 1979) and the demonstration that 'DI RNPs' have specific interfering properties (Janda & Nayak, 1979). The conflicting data might be resolved if the proportion of subgenomic RNAs present in DI virus is related quantitatively to their ability to cause interference, but before accepting this we would need to know more about the molecular mechanism of interference and indeed the reason why certain genome segments persist.

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REFERENCES


Short communications


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