Characterization of Bunyamwera virus defective interfering particles

Arvind H. Patel1,2* and Richard M. Elliott2

1Medical Research Council Virology Unit and 2Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR, U.K.

In an attempt to isolate conditional lethal amber nonsense mutants of Bunyamwera virus, five variants were found which produced small plaques on BHK and mouse L cells. Characterization of these variants by Northern blotting showed that they synthesized defective (subgenomic) RNAs derived from the L RNA segment. No subgenomic M or S segment RNAs were detected. The defective L RNAs were shown to be packaged into virus particles, and four of five preparations caused interference with the multiplication of standard virus. When defective-containing preparations were mixed with standard virus and grown in doubly infected cells a reduction in titre of standard virus of up to 400-fold was observed. Hence these preparations most probably contained defective interfering (DI) particles. Novel DI-specific polypeptides were synthesized in DI virus-infected cells. These novel proteins could be precipitated by antisera raised against either the N or C terminus, or both, of the L protein. Nucleotide sequence analysis of cloned cDNA to prominent DI RNAs in three different defective virus preparations revealed that the DI RNA in each case had suffered a single internal deletion of the L segment while retaining the 5'- and 3'-terminal sequences. The extent of the deletion ranged between 72% and 77% of the L RNA segment. Our results suggest that these DI particles may have arisen during the attempted isolation of Bunyamwera virus amber mutants on mouse L cells, since defective/subgenomic RNAs derived from the L and M segments were readily generated in mouse L cells but not in BHK cells, following infection with wild-type virus.

Introduction

Defective interfering (DI) particles are viral particles that contain only a fraction of the genome of parental virus and most likely arise by an aberrant replication event. DI particles require the presence of a homologous helper virus to replicate their defective genome, and in turn interfere with the replication of the helper virus. Probably most groups of RNA and DNA viruses generate DI particles, and those of negative-strand viruses such as vesicular stomatitis virus (VSV), Sendai virus and influenza viruses have been particularly well studied (reviewed by Lazzarini et al., 1981; Nayak et al., 1989; Holland, 1990).

The family Bunyaviridae is a large group of mainly arthropod-transmitted viruses that contain a tripartite ssRNA genome. The three genome segments are designated L (large), M (medium) and S (small) and are of negative sense polarity or, for some S segments, ambisense polarity (reviewed by Elliott, 1990). There are few reports on Bunyaviridae DI particles. Kascsak & Lyons (1978) generated particles which interfered with the multiplication of homologous virus by high multiplicity passage of Bunyamwera virus in BHK cells, and showed that these particles contained S segment-sized RNA. Later Elliott & Wilkie (1986) reported that particles were shed from Aedes albopictus (mosquito) cells persistently infected with Bunyamwera virus that contained only S segment sequences, and had interfering activity. Neither of these cases represents 'classical' DI particles. More typical DI particles were inferred from the observation by Cunningham & Szilagyi (1987) of subgenomic RNAs derived from the L segment in purified preparations of Germiston bunyavirus. Recently we demonstrated the presence of defective L segment RNAs in A. albopictus cells persistently infected with Bunyamwera virus, but these shorter RNAs were not packaged into particles (Scallan & Elliott, 1992).

In the course of experiments to isolate amber nonsense mutants of Bunyamwera virus, following the successful regimen described by White & McGeoch (1987) to obtain amber nonsense mutants of VSV, variants were found which produced small plaques on BHK cells. Characterization of the RNAs and proteins synthesized by these variants indicated they contained DI particles. The results of these analyses are presented here.
Methods

Cells, viruses and radioisotopic labelling. Bunyamwera virus and the DI isolates were grown and titrated on baby hamster kidney (BHK) cells as described by Watret et al. (1985). The putative amber mutants of 5-fluorouracil-treated Bunyamwera virus were isolated on the L39 cell line (Hudziak et al., 1982), which expresses amber suppressor tRNA (Su+), as described by White & McGeoch (1987). The parental LMTK- cell line used was Su+. Radiolabelling of infected cells was performed as detailed in Watret et al. (1985). At the end of the labelling period, the cells were washed with PBS, harvested by scraping, lysed by repeated freezing and thawing and analysed, either directly or after immunoprecipitation, on a polyacrylamide gel (Laemmli, 1970). All immunoprecipitations were performed as described by Zweig et al. (1980). The antisera used for immunoprecipitations were raised against either the N- or the C-terminal portions of the Bunyamwera virus-encoded L protein fused to bacterial β-galactosidase (H. Jin & R. M. Elliott, unpublished data).

Isolation and analysis of viral RNAs. Total cellular RNA was extracted from infected tissue culture cells at 48 h post-infection (p.i.) as described by Chomczynski & Sacchi (1987). Encapsidated viral RNA was extracted using the same method from intracellular viral nucleocapsids which were isolated from infected cells as described by Leppert et al. (1979). For Northern blot analysis, the RNA was fractionated on a 1.2%; agarose-formaldehyde gel and transferred to Hybond-N membrane (Amersham) according to Maniatis et al. (1982). Hybridization was performed using 32P-labelled probes at 42 °C for 18 h in a buffer composed of 50% formamide, 5 x saline–sodium phosphate–EDTA, 2 x Denhardt’s reagent and 0.1 mg/ml calf thymus DNA. The probes were essentially full-length cDNAs to the L, M and S segments cloned into plasmid vectors (Scallan & Elliott, 1992). Filters were washed for 15 min at room temperature, twice with 2 x SSC, 0.1% SDS, and twice with 0.1 x SSC, 0.1% SDS.

cDNA synthesis and PCR. Oligonucleotides representing nucleotides (nt) 117 to 136 (APS) and 30 to 49 (AP7) (5’ end, positive strand), and the complementary sequences (genomic, negative sense) from nt 6720 to 6701 (AP6) and 6849 to 6830 (AP8) (5’ end) of the Bunyamwera virus L genomic RNA segment (Elliott, 1989) were synthesized as primers for cDNA synthesis and for amplification by the polymerase chain reaction (PCR) of encapsidated defective L RNA segments of DI preparations M3, M5 and M7. The cDNA PCR was performed as described by Kawasaki & Wang (1989) using avian myeloblastosis virus reverse transcriptase (Life Sciences) and Taq DNA polymerase (Perkin-Elmer Cetus). The amplification reaction was performed in an IHR 2024 thermal cycler (Eppendorf) for 30 cycles of denaturation at 95 °C for 10 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 2 min.

Cloning of amplified cDNAs. All DNA manipulations were performed by standard procedures (Maniatis et al., 1982). The cDNA PCR products were extracted with phenol/CHCl3 and precipitated with ethanol. The ends were made blunt using Klenow polymerase and T4 DNA polymerase followed by phosphorylation with T4 polynucleotide kinase. The blunt-ended cDNAs to the defective L segment RNA from DI isolates M3, M5 and M7 were cloned into Smal-cleaved plasmid pTZ19U (Pharmacia) to yield plasmids pM35, pM5 and pM78 respectively.

Nucleotide sequencing. Overlapping fragments of cloned cDNA inserts were generated either by sonication (pM35 and pM5; Bankier et al., 1987) or by restriction enzyme digestions (pM78), cloned into the M13 vector M13mp18, and sequenced by the dideoxynucleotide chain termination method of Sanger et al. (1980). Nucleotide sequence data were read into a computer. Random data were assembled into contiguous sequences and analysed using programs in the University of Wisconsin Genetics Computer Group (UWCGC) package (Devereux et al., 1984).

Results

Isolation of Bunyamwera virus variants

Attempts were made to isolate conditional lethal amber nonsense mutants of Bunyamwera virus, following the successful strategy adopted by White & McGeoch (1987) to obtain amber nonsense mutants of VSV. Five putative mutants were isolated from a 5-fluorouracil-treated Bunyamwera virus stock by their ability to grow on mouse L39 cells, which express a Xenopus laevis amber suppressor tRNA (Su+) cells, but not on the non-suppressor (Su-) parental LMTK- cells. However, further analysis of these variants showed that the host restriction on Su- cells was cell growth cycle-dependent; the titres of the variants on freshly grown Su- cells were comparable to those on Su+ cells, but the growth restriction occurred only on ageing (6 days after plating at 31 °C)Su- cell monolayers. Moreover the growth of the variants on BHK (as compared to L39) cells was not restricted. The variants, named M2, M3, M4, M5 and M7, displayed a small plaque phenotype as compared to the wild-type Bunyamwera virus on BHK as well as L39 and LMTK- cells (data not shown).

Characterization of viral RNAs

The RNA species in infected BHK cells were analysed by Northern blot hybridization with cloned cDNA probes. Total cellular RNAs extracted from infected BHK cells were probed with essentially full-length cloned cDNAs derived from the L, M or S genomic RNA segments of Bunyamwera virus. As shown in Fig. 1, the L-specific DNA probe hybridized not only to standard length L RNA (6.9 kb), but also to a number of smaller RNA species migrating in the range of approximately 1.5 to 2.9 kb in RNA extracted from cells infected with the variants. Only the standard length L segment was readily detected in the RNA preparation from wild-type virus-infected cells. When total RNA from cells infected with the variants or with wild-type virus was probed with either the M- or the S-specific cDNA, only the standard length RNAs were detected (Fig. 1). These results indicate that the variants contained defective RNAs derived from the L RNA segment. Interestingly, in M2-, M3- and M7-infected cells, the full-length L segment was less abundant than the defective RNAs (Fig. 1). The L segment-specific defective RNAs were retained in the virus population after multiple passages in BHK cells (not shown).
We next studied the encapsidation of the defective RNAs by hybridization of RNA isolated from purified viral nucleocapsids with the L-specific cDNA. As shown in Fig. 2, the defective RNAs derived from the L segment could be detected in nucleocapsids of all five defective virus preparations, indicating that they were efficiently packaged. The relative amounts of full-length compared to defective L RNA packaged varied between the different preparations, and did not always reflect the abundance of these RNAs seen in analysis of the total cellular RNA (Fig. 1). As expected, only the standard length L RNA segment was present in the wild-type virus nucleocapsids (Fig. 2).

In order to ascertain whether the generation of defective RNAs in Bunyamwera virus was cell-type-dependent, we infected both BHK and LMTK− cells with the stock of wild-type virus at various m.o.i. Intracellular RNAs were analysed by Northern blotting. In LMTK− cells infected at an m.o.i. of 0.01, 0.1, 1.0 or 10.0 several low M, RNA species derived from the L and M segments were detected but only the standard length S segment was observed (Fig. 3a, b and c). In contrast, RNA extracted from infected BHK cells contained only the standard length L, M and S RNAs (Fig. 3d, e and f). These results suggest that the subgenomic RNAs detected in all the defective virus preparations were probably generated during the initial attempts to isolate amber nonsense mutants of Bunyamwera virus in mouse L cells.

**Effects of the defective viruses on the replication of standard Bunyamwera virus**

We subsequently investigated whether the defective viruses were able to interfere with the replication of the wild-type Bunyamwera virus. BHK cells were co-infected with wild-type virus and different amounts of the defective virus preparations. Following incubation at 31 °C for 48 h, infectious virus yields were determined by plaque assay on BHK cells (Table 1). Co-infection of cells with the standard virus and four out of five defective virus preparations resulted in a marked decrease of virus yields compared to that of standard virus alone. The degree of interference was dependent on the amount of defective virus preparation added. The level of interference was most striking in cells co-infected with the standard virus and DI preparations M2 and M7 which, compared to that of the standard virus alone, reduced the yield of virus to 0.97% and 0.2% (i.e. 94- and 400-fold reduction) respectively. The virus preparation M4 failed to interfere with the replication of the standard virus (Table 1). The most likely interpretation of these data is
that interference is mediated by the defective RNAs, but definitive proof requires removal of all wild-type virus from the defective virus preparation, e.g. physically by centrifugation or by selective u.v. inactivation. Thus far such experiments have proved difficult.

Analysis of viral polypeptides in infected cells

The synthesis of viral polypeptides in BHK cells infected with the wild-type virus and the DI particle preparations was examined. Infected cells were labelled with [35S]methionine for 18 h at 52 h p.i. The results show that all five DI particle preparations synthesized proteins corresponding to Bunyamwera virus proteins L, G1 and N (Fig. 4). Interestingly, the DI particle preparations also produced novel polypeptides of various sizes in infected cells. Similar observations were also made in infected L39 and LMTK− cells (not shown). Thus, virus stocks M2, M3, M4, M5 and M7 gave rise, respectively, to polypeptides of M, 82.5K, 75K, 70K, 86.5K and 27K
Bunyamwera virus DI particles

Fig. 4. SDS–PAGE analysis of the polypeptides synthesized in infected cells. BHK cells were mock-infected (g) or infected with wild-type Bunyamwera virus (f) or DI isolates M2, M3, M4, M5 or M7 (a to e) at 1 p.f.u. per cell. Polypeptides were labelled with [35S]methionine, and cell extracts were analysed either directly (lanes 1) or after immunoprecipitation (lanes 2 and 3) with antisera raised against either the N (lanes 2) or the C (lanes 3) terminus of the L protein on a 10% polyacrylamide gel. The positions of Bunyamwera virus L, G1 and N proteins are indicated. Arrowheads indicate the positions of the DI-specific polypeptides. The migration of protein size markers is shown at the right. The reactivity of anti-C-terminal L protein serum with N protein is thought to be due to immunoprecipitation of nucleocapsids which are not fully dissociated.

in infected cells (Fig. 4). We used two rabbit antisera raised against portions of the L protein of Bunyamwera virus (see Methods) in order to establish that the DI-specific polypeptides were of L protein origin. One was raised against amino acids 67 to 406 representing the N terminus of L, and the other against amino acids 2001 to 2214 representing the C terminus (H. Jin & R. M. Elliott, unpublished data). Both antisera were used to test their ability to precipitate the novel polypeptides as well as the full-length L protein from extracts of infected cells. As shown in Fig. 4, both antisera specifically precipitated the 82.5K and 75K polypeptides synthesized by the DI-M2 and DI-M3 particle preparations respectively, whereas the 86.5K polypeptide synthesized by DI-M5 was precipitated by the C terminus-specific antiserum only. The 70K polypeptide synthesized by DI-M4 reacted weakly with the N terminus-specific antiserum. The 27K polypeptide produced by DI-M7 did not react with either of the antisera. A number of minor polypeptides in DI-M2- and DI-M3-infected cells were also precipitated by the C terminus-specific antiserum. These results suggested that the novel polypeptides encoded by at least four of the five DI viruses were produced from DI-specific L mRNAs. They also
Fig. 5. Diagram of the structures of the defective L segment RNAs of DI isolates M3, M5 and M7. The full-length L segment of standard Bunyamwera virus (wt BUN L) is presented at the top and the defective RNAs below. Deleted regions (---) in the defective L sequence and the relevant nucleotide positions are indicated. The nucleotide sequence and the deduced amino acids flanking the deletions are shown, underlined letters indicating a shift in the translational reading frame. Stop codons are indicated by an asterisk. The relevant amino acids are numbered. Nucleotide substitutions in DI RNAs compared with the BUN L sequence are indicated by lower case letters.

Table 1. Interference of defective Bunyamwera (BUN) viruses with the multiplication of standard Bunyamwera virus

<table>
<thead>
<tr>
<th>Virus inoculum</th>
<th>Multiplicity of infection</th>
<th>Yield of virus (p.f.u./ml)</th>
<th>Yield of standard virus (%)</th>
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<tr>
<td>Standard BUN alone</td>
<td>1-0</td>
<td>3.2 x 10⁷</td>
<td>100-0</td>
</tr>
<tr>
<td>Standard BUN + M2</td>
<td>1-0 + 0-01</td>
<td>8.5 x 10⁶</td>
<td>26-6</td>
</tr>
<tr>
<td>Standard BUN + M2</td>
<td>1-0 + 0-1</td>
<td>3.6 x 10⁵</td>
<td>1-13</td>
</tr>
<tr>
<td>Standard BUN + M2</td>
<td>1-0 + 1-0</td>
<td>3.1 x 10⁴</td>
<td>0-97</td>
</tr>
<tr>
<td>M2 alone</td>
<td>1-0</td>
<td>1-3 x 10⁵</td>
<td>-</td>
</tr>
<tr>
<td>Standard BUN + M3</td>
<td>1-0 + 0-01</td>
<td>2.5 x 10⁷</td>
<td>78-1</td>
</tr>
<tr>
<td>Standard BUN + M3</td>
<td>1-0 + 0-1</td>
<td>4.0 x 10⁶</td>
<td>12-5</td>
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<tr>
<td>Standard BUN + M3</td>
<td>1-0 + 1-0</td>
<td>1-1 x 10⁵</td>
<td>3-4</td>
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<td>1-0</td>
<td>1-8 x 10⁵</td>
<td>-</td>
</tr>
<tr>
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<td>1-0 + 0-01</td>
<td>4.5 x 10⁷</td>
<td>14-1</td>
</tr>
<tr>
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<td>1-0 + 0-1</td>
<td>2.5 x 10⁷</td>
<td>78-0</td>
</tr>
<tr>
<td>Standard BUN + M4</td>
<td>1-0 + 1-0</td>
<td>3.6 x 10⁷</td>
<td>110-9</td>
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<td>1-0</td>
<td>7-0 x 10⁶</td>
<td>-</td>
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<td>1-0 + 0-01</td>
<td>1-6 x 10⁷</td>
<td>50-0</td>
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<td>1-5 x 10⁶</td>
<td>4-7</td>
</tr>
<tr>
<td>Standard BUN + M5</td>
<td>1-0 + 1-0</td>
<td>3-8 x 10⁵</td>
<td>1-2</td>
</tr>
<tr>
<td>M5 alone</td>
<td>1-0</td>
<td>4-9 x 10⁵</td>
<td>-</td>
</tr>
<tr>
<td>Standard BUN + M7</td>
<td>1-0 + 0-01</td>
<td>3-5 x 10⁷</td>
<td>107-8</td>
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<tr>
<td>Standard BUN + M7</td>
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<td>8-9</td>
</tr>
<tr>
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<td>6-5 x 10⁴</td>
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<td>M7 alone</td>
<td>1-0</td>
<td>1-4 x 10⁵</td>
<td>-</td>
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suggested that the L gene had undergone internal deletions, which in the case of 82.5K and 75K polypeptides of DI-M2 and DI-M3 were probably in frame.

cDNA synthesis and sequence analysis

The full nucleotide sequence of the L RNA segment of Bunyamwera virus is known (Elliott, 1989). Based on this sequence information, we used oligonucleotide AP5 complementary to the 5' end of the L segment to prime cDNA synthesis using encapsidated defective L RNAs of DI particle preparations M3 and M7 as templates. The cDNA was amplified using primer AP5 and another primer (AP6) which was complementary to the 3' end of the positive strand of the L segment (see Methods). Similarly, primers AP7 and AP8 were used to synthesize and amplify cDNA to encapsidated defective L RNA of the DI particle preparation M5. This procedure yielded single cDNA products of 1-3, 1-55 and 1-75 kb corresponding to the major DI RNAs present in M3, M5 and M7 nucleocapsids respectively. Following end repair, the cDNAs were cloned directly into the SmaI
site of plasmid pTZ19U and the complete nucleotide sequence of each of the PCR products was determined.

Sequence analysis revealed that the DI RNA in each case had suffered a single internal deletion, while retaining the 5' and 3' termini of the L segment (Fig. 5). In DI-M3 RNA a 5293 bp fragment from nt 804 to 6097 (i.e. 77% of the RNA segment) was deleted, and in DI-M5 RNA the deletion spanned nt 270 to 5481, which represents 76% of the L segment. The DI-M7 RNA suffered a deletion of 4959 bp fragment from nt 700 to 5659, which corresponds to 72% of the L segment. The sequenced RNAs of DI-M3, DI-M5 and DI-M7 could encode truncated L products of M, 30K, 11K and 26.5K respectively. The deletions occurred in such a manner that the predicted ORFs in DI-M3, DI-M5 and DI-M7 RNAs, respectively, contained the N-terminal 251, 73 and 215 amino acids of L, followed by 8, 21 and 10 codons not present in the authentic L ORF. We could not detect the presence, in infected cells, of DI-specific polypeptides corresponding to the predicted ORFs of DI-M3 and DI-M5 RNAs; however, a polypeptide of approximately 27K was detected in DI-M7-infected cells, which may be encoded by the predicted truncated L ORF of DI-M7 RNA (Fig. 4).

Sequence analysis also revealed single nucleotide substitutions at positions 427 (T to C) and 6410 (A to G) in the defective L RNA of M3, and at position 6340 (T to C) in that of M7. The fact that these are transition mutations suggests that they may have arisen following treatment with 5-fluorouracil (a mutagen thought to produce transition mutations) which was initially used in the attempted isolation of amber mutants of Bunyamwera virus. Alternatively, substitution mutations may have arisen due to incorporation of incorrect nucleotides by the Taq polymerase during the PCR reaction.

Discussion

In an attempt to isolate amber mutants of Bunyamwera virus using the successful strategy adopted by White & McGeoch (1987) to obtain amber nonsense mutants of VSV, five variants were found which produced small plaques on BHK and other tissue culture cells. The following observations strongly indicate that the variants contained DI particles. Northern blot analysis of RNA isolated from BHK cells infected with the variants showed the presence of a number of low M, RNA species derived from the Bunyamwera virus L segment as well as the standard length L RNA. No subgenomic M or S segment RNAs were detected in the defective virus-infected cells. The defective L RNAs were efficiently packaged into virus particles. Four of five defective virus preparations interfered to varying degrees with the replication of standard virus, although unequivocal proof that interference was due to the deleted RNAs was not obtained.

In the Bunyaviridae, the L protein, which is thought to be the virion-associated transcriptase or RNA polymerase, and the nucleocapsid protein are tightly associated with each of the three genome and antigenome RNAs to form nucleocapsids (Obijeski et al., 1976). The 3' and 5' ends of the Bunyamwera virus RNAs are conserved and complementary (Elliott, 1990), and it has been suggested that the conserved terminal sequences of the RNAs, in particular the 5' end, are important for encapsidation by the nucleocapsid protein (Raju & Kolakofsky, 1987, 1989). Our observations that the DI RNAs of all five DI particles are efficiently packaged indicate that the encapsidation signals have been retained in the DI RNAs. The small plaque phenotype displayed by the DI particles and the maintenance of defective RNAs with passage suggest that the DI RNAs may be co-packaged with the standard length RNA segments into virus particles.

BHK cells infected with the DI virus preparations contained DI-specific polypeptides of various sizes. The DI-specific polypeptides synthesized by DI isolates M2, M3, M4 and M5 were of L protein origin as demonstrated by their reactivity with antisera raised against the N and C termini of the L protein. This suggested that the defective L RNA segments of the DI particles were internally deleted, and that those encoding the polypeptides of M, 85K (DI-M2) and 75K (DI-M3) may carry deletions that are in frame since these polypeptides were precipitated by both antisera. We were unable to detect either of these proteins in the DI particle preparations (data not shown).

Nucleotide sequence analysis of the major encapsidated DI RNA in M3, M5 and M7 isolates showed that each was derived by a single internal deletion event in the standard L segment of Bunyamwera virus which removed 72 to 77% of the gene. The retention of the 5' and 3' termini in the DI RNAs was not surprising as these regions are likely to be involved in RNA polymerase binding and initiation of replication. It is not known whether the predicted truncated L ORFs encoded by defective L RNAs of DI-M3 and DI-M5 are translated into proteins as these truncated polypeptides were not detected in infected cells. The 75K and 86-5K polypeptides synthesized by DI-M3 and DI-M5 isolates, respectively, are likely to be encoded by one of the minor defective RNAs present in these preparations. The DI RNA of DI-M7 could encode a truncated L protein of 26-5K, containing a translational shift at amino acid 215. A 27K polypeptide was synthesized in large amounts in DI-M7-infected cells, although it was not precipitated efficiently (if at all) by the N-terminal antiserum. It is
possible that the antibody recognition epitope in the N-terminal fusion protein used to immunize rabbits occurs between amino acids 215 and 406, a region lost with the deletion in DI-M7 RNA. Another possibility is that the 27K polypeptide is encoded by a minor defective RNA of DI-M7 isolate.

The molecular characterization of Bunyamwera virus DI particles presented in this report is consistent with the 'classical' pattern of DI viruses (Huang & Baltimore, 1970) in that they carry large internal deletions representing essential portions of the genomic L segment and therefore require homologous helper virus in order to replicate, the defective RNAs are packaged into virus particles, and the DI particles interfere with the replication of the homologous helper virus in doubly infected cells. The subgenomic DI RNAs of DI-M3, DI-M5 and DI-M7 can be assigned to the 5'3' (internal deletion type) class of DI RNAs (Lazzarini et al., 1981). DI particles of VSV and influenza virus belonging to this class, and which also carry deletions in genes specifying polymerase function, have been described (Epstein et al., 1980; Davis et al., 1980; Moss & Brownlee, 1981), but our data are the first reported for any member of the Bunyaviridae.

The mechanism of the generation of Bunyamwera virus defective RNAs is not understood. We were unable to detect subgenomic/DI RNAs in BHK cells infected with standard Bunyamwera virus even at high m.o.i. (10 p.f.u./cell). This is in contrast to the observations of Kascak & Lyons (1978) that the BHK cell line displays 'high autointerference' with the replication of Bunyamwera virus. Interestingly, our results show that Bunyamwera virus defective RNAs (and presumably therefore DI particles) can be generated relatively easily even at a low m.o.i. (0-01 p.f.u./cell) in mouse L cells in a single cycle of infection with standard virus.

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