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Characterization of Maguari orthobunyavirus mutants suggests the nonstructural protein NSm is not essential for growth in tissue culture

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Abstract

Maguari virus (MAGV; genus *Orthobunyavirus*, family Bunyaviridae) contains a tripartite negative-sense RNA genome. Like all orthobunyaviruses, the medium (M) genome segment encodes a precursor polyprotein (NH_2-Gn-NSm-Gc-COOH) for the two virion glycoproteins Gn and Gc and a nonstructural protein NSm. The nucleotide sequences of the M segment of wild-type (wt) MAGV, of a temperature-sensitive (ts) mutant, and of two non-ts revertants, R1 and R2, that show electrophoretic mobility differences in their Gc proteins were determined. Twelve amino acid differences (2 in Gn, 10 in Gc) were observed between wt and ts MAGV, of which 9 were maintained in R1 and R2. The M RNA segments of R1 and R2 contained internal deletions, resulting in the removal of the N-terminal 239 residues of Gc (R1) or the C-terminal two thirds of NSm and the N-terminal 431 amino acids of Gc (R2). The sequence data were consistent with analyses of the virion RNAs and virion glycoproteins. These results suggest that neither the N-terminal domain of Gc nor an intact NSm protein is required for the replication of MAGV in tissue culture.

Keywords: Bunyavirus; Maguari virus; Nonstructural protein; Glycoprotein

Introduction

The family Bunyaviridae contains over 300 enveloped viruses that have tripartite single-stranded negative-sense RNA genomes. The viruses replicate in the cytoplasm, and virion maturation usually occurs by budding at membranes of the Golgi apparatus. The family is classified into five genera: *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus* (Nichol et al., 2005). Bunyamwera virus is the prototype of the family and is contained in the *Orthobunyavirus* genus. Orthobunyaviruses encode four structural and two nonstructural proteins in their genomes: the L (large) RNA segment encodes the L protein, an RNA-dependent RNA polymerase; the M (medium) RNA segment encodes the two virion glycoproteins, Gn and Gc, and a nonstructural protein, NSm, as a polyprotein precursor that is co-translationally cleaved; and the S (small) RNA segment encodes the nucleocapsid (N) protein and a second nonstructural protein, NSs, in overlapping reading frames (reviewed by Bishop, 1996; Nichol, 2001; Schmaljohn and Hooper, 2001).

Viruses in the *Orthobunyavirus* genus are divided among 18 serogroups (Calisher, 1996). Temperature-sensitive (ts) mutants of three representatives of the Bunyamwera serogroup, Batai (BATV), Bunyamwera (BUNV), and Maguari (MAGV) viruses were isolated by Iroegbu and Pringle (1981) following treatment with 5-flourouracil and have been used to investigate genetic reassortment between different viruses (reviewed by Pringle, 1996). Mutants assigned to group II were shown to have the ts lesion in the M segment (Iroegbu and Pringle, 1981; Pringle et al., 1984). In screening progeny from a recombination experiment, it was observed that reversion from the ts to non-ts phenotype was frequently associated with a change in the electrophoretic mobility of the Gc protein of the MAGV parent. (Note that in older literature the Gc protein is called G1 and Gn is called G2.) Further work showed that 35 of 36 non-ts revertants of MAG ts8 showed this phenotype, and

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electrophoretic mobility changes in Gc were also observed for non-ts revertants of two other group II mutants, MAG ts14 and ts45. In contrast, no changes in Gc mobility were observed in non-ts revertants of BATV or BUNV group II mutants (Elliott et al., 1984). The non-ts revertants of MAG ts8 fell into two classes, those whose Gc protein had an apparent molecular weight of 80 kDa (designated R1) and those whose Gc protein had an apparent molecular weight of 60 kDa (designated R2); the Gc protein of wild-type (wt) MAGV is about 110 kDa (Elliott et al., 1984; Murphy and Pringle, 1987). Murphy and Pringle (1987) further showed that there were differences in the degree of glycosylation of the faster migrating forms of Gc synthesized by MAG R1 and R2, compared to that of the wt Gc, but these differences were insufficient to account for the observed electrophoretic mobility differences.

To characterize these MAG viruses further, we have cloned and sequenced the M genome segments of wt MAGV, MAG ts8, and two non-ts revertants, MAG R1 and MAG R2. The sequence data show that MAG R1 and MAG R2 contain internal deletions in their M segments that result in deletion of the N-terminal region of Gc and further that in MAG R2 the NSm coding region is severely truncated.

Results

Analysis of Maguari virus proteins

Stocks of the MAG viruses for use in this study were grown in BHK cells at 33 °C following low multiplicity infection, and titers of virus released into the supernatant ranged from 3.3×10^7 (MAG R1) to 1.2×10^8 (wt MAGV) pfu/ml. The temperature-sensitive phenotype of MAGts was confirmed by measuring the yields of virus from BHK cells, infected at 5 pfu/cell, 48 h after infection at either 33 °C or 38 °C; similar yields for all viruses were obtained at 33 °C, but at 38 °C, the titer of MAG ts8 was at least 1000-fold lower than that of wt MAGVV or the revertant viruses MAG R1 and MAG R2 (data not shown). The protein profiles of the MAG viruses were analyzed by polyacrylamide gel electrophoresis of immunoprecipitated radiolabeled cell extracts (Fig. 1A). Antisera were raised in two rabbits (designated 628 and 629) against purified wt MAGV particles, and hence only the structural proteins would be detected; both antisera reacted similarly in immunoprecipitation. It was difficult to observe the L protein band, but, on longer exposure of the gel (data not shown), no differences in its electrophoretic mobility were observed between the different MAG viruses; likewise, the Gn and N proteins of the four viruses migrated similarly. Both wt MAGV and MAG ts8 had Gc proteins of about 110 kDa, but the non-ts revertant viruses MAG R1 and MAG R2 had Gc proteins of 80 kDa and 60 kDa, respectively, in agreement with previous observations (Murphy and Pringle, 1987). Radiolabeled virion preparations were also examined by PAGE (Fig. 1B). The four structural proteins were detected, and, again, the mobilities of the L, Gn, and N proteins were similar. However, the revertant viruses R1 and R2 contained the shorter 80 kDa and 60 kDa Gc proteins, respectively, compared to wt and ts8 viruses which contained the full-size Gc of 110 kDa. This indicated that the shorter Gc proteins observed in infected cell extracts are indeed incorporated into released virus particles.

Cloning and sequence determination of Maguari virus M segment cDNAs

The M RNA segments of the MAG viruses were cloned as cDNA via a reverse transcription-PCR protocol as described in Materials and methods. Preliminary sequence analysis of the wt MAGV M segment (data not shown) enabled the design of primers for specific reverse transcription and subsequent nested PCR for the full-length M segment as described. Using this

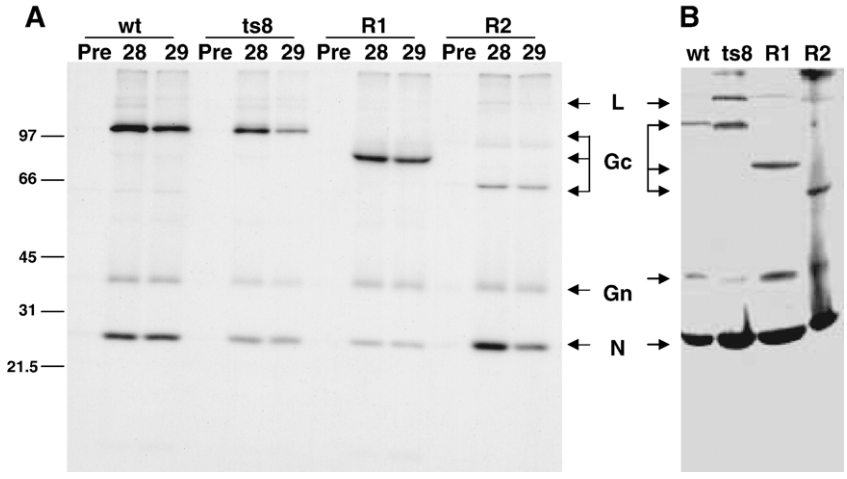

Fig. 1. Analyses of Maguari virus proteins. (A) Immunoprecipitation of radiolabeled infected cell extracts. BHK cells were infected with the different MAG viruses and radiolabeled cell extracts prepared as described in Materials and methods. The extracts were reacted with pre-immune rabbit serum (Pre) or immune serum from rabbits 628 (28) or 629 (29) and precipitated proteins fractionated by SDS–PAGE. The positions of the viral structural proteins L, Gc, Gn, and N are shown. The positions of molecular weight standards are indicated on the left. (B) Radiolabeled virion preparations. The supernatants containing virus particles from radiolabeled infected cells were collected by centrifugation as described in Materials and methods and analyzed by SDS–PAGE. The positions of the viral proteins are indicated. Note that more material was loaded on the MAG ts8 track, giving rise to artefactual faster migration of Gc and Gn compared to wt virus.