RNA SYNTHESIS BY GERMISTON VIRUS IN INFECTED CELLS 56

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We analysed by agarose gel electrophoresis the RNA species synthesised during high multiplicity of infection of BHK 21 cells with Germiston virus, a member of the Bunyamwera family. Altogether seven RNA species were synthesised for varying periods of time during the eight hours of incubation. The individual RNA species were characterised by blot hybridisation using cDNA clones of the virion RNA species. The roles of the various RNA species in virus development were studied by means of in vitro protein synthesis and microinjection into Xenopus oocytes.

CHARACTERIZATION OF TEMPERATURE SENSITIVE MUTANTS OF PICHINDE VIRUS 57

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The genome of Pichinde virus consists of 2 segments of single-stranded RNA. The L RNA segment encodes a large protein thought to be an RNA polymerase while the S RNA segment encodes 2 proteins. It is postulated that the nucleoprotein (N) is encoded in a negative sense at the 3' end of S RNA while the glycoprotein precursor (GPC) is encoded in a positive sense at the 5' end. The implications of this coding strategy is that mRNA for N could be transcribed from parental RNA while mRNA for GPC would have to be transcribed from complementary RNA. To investigate the replicative process of Pichinde virus in greater detail, we have characterized 14 temperature sensitive (ts) mutants of Pichinde virus. Viral protein (N and GPC) synthesis was examined by incubating infected cells in the presence of [^{35}S]methionine, immunoprecipitating viral proteins from lysates of the cells and resolving the precipitated proteins by polyacrylamide gel electrophoresis. The presence of viral glycoproteins on the surface of infected cells was assessed by immunofluorescent staining of viable cells. The mutants appeared to fall into 4 groups defined by phenotypic properties. One group synthesized reduced quantities of N and no GPC at the nonpermissive temperature. A second group synthesized N and increased amounts of GPC at the nonpermissive temperature but no antigens were found on the cell surface. A third group synthesized reduced quantities of both N and GPC at both temperatures and had abnormal cell surface staining. The fourth group synthesized normal quantities of both N and GPC at the nonpermissive temperature and the usual pattern of cell surface fluorescence was found. The implications with respect to the ts defects producing these phenotypes will be discussed.