JOURNAL OF VIROLOGY, Oct. 1977, p. 397-400

Vol. 24, No. 1
Printed in U.S.A.

NOTES

Bunyamwera Virus-Induced Polypeptide Synthesis

T. H. PENNINGTON,* C. R. PRINGLE, AND M. A. McCRAE

Department of Virology and Medical Research Council Virology Unit, Institute of Virology, University of Glasgow, Glasgow 911 5JR, Scotland

Received for publication 8 March 1977

Bunyamwera virus-induced polypeptide synthesis in BSC-1 cells has been studied using polyacrylamide gel electrophoresis and autoradiography. Four virus-induced polypeptides were identified. Their molecular weights were 200 × 10^6 (L), 128 × 10^6 (G1), 31 × 10^6 (G2), and 23 × 10^6 (N). Pulse-chase experiments, short labeling experiments, and experiments using amino acid analogs failed to show evidence of polypeptide processing by proteolytic cleavage. Analysis of the kinetics of synthesis of these polypeptides showed that a clear division into early and late categories could be made, the onset of synthesis of polypeptide N preceding that of polypeptides G1 and G2. The rate of synthesis of polypeptides N and L rapidly reached a peak and then declined. Polypeptides G1 and G2 were made for several hours; their rate of synthesis then declined. All four polypeptides then continued to be made in relatively small amounts for many hours.

Bunyamwera virus was first isolated from *Aedes* mosquitoes captured in Semliki Forest, Uganda (13). It is the type species of the arbovirus genus Bunyavirus and of the family *Bunyaviridae*, which includes more than 80 different viruses serologically related to the type virus (reviewed in 10). Recent studies on the virions of Bunyaviruses (1, 7, 8, 10) indicate that they contain single-stranded RNA, probably in three segments, and that they are enveloped with two surface glycoproteins. The internal ribonucleoprotein contains two polypeptides, one of high molecular weight.

This paper reports the results of studies on the kinetics of virus-induced polypeptide synthesis in BSC-1 cells infected with Bunyamwera virus. Experiments designed to investigate polypeptide processing are also described.

Bunyamwera virus (Smithburn strain) was obtained from J. S. Porterfield and cloned by three sequential isolations from single plaques. Virus stocks were grown from individual plaques in BSC-1 cells to prevent the accumulation of defective interfering particles.

The time course of virus-induced polypeptide synthesis was followed in experiments where infected cells were pulse-labeled with [^{35}S]methionine at various times after infection. An autoradiogram of 8% acrylamide gels is shown in Fig. 1. Similar results were obtained using gels of higher and lower acrylamide concentrations and 5 to 15% gradient gels. Four virus-induced polypeptides were identified. Using vesicular stomatitis virus (Indiana serotype), Sendai virus, and reovirus type 3 polypeptides as markers, the molecular weights of these polypeptides were estimated to be 200 × 10^3, 128 × 10^3, 31 × 10^3, and 23 × 10^3. As these molecular weights were almost identical to those of the four structural polypeptides of La Crosse virus, which is serologically related to Bunyamwera virus (10), the nomenclature adopted for the La Crosse virus polypeptides (7) was applied to the Bunyamwera virus-induced polypeptides. Thus, the largest of these polypeptides was termed L; the smallest, N; and the two intermediate-sized polypeptides, G1 (128 × 10^3 daltons) and G2 (31 × 10^3 daltons). As polypeptide L was synthesized in only small amounts and as it comigrates in gels with at least one host polypeptide, we only tentatively identify it as a virus-induced polypeptide.

These virus-induced polypeptides could be divided into early and late categories according to their rates of synthesis at various times during the virus growth cycle (Fig. 2). Thus, the synthesis of polypeptide N was first detected at 2 h postinfection (p.i.). Its rate of synthesis then increased, reaching a peak at about 4 h p.i.; the rate subsequently declined. On the other hand, the synthesis of polypeptide G1 started late in the virus growth cycle, at about 4 h p.i. The rate of synthesis of this polypeptide was then

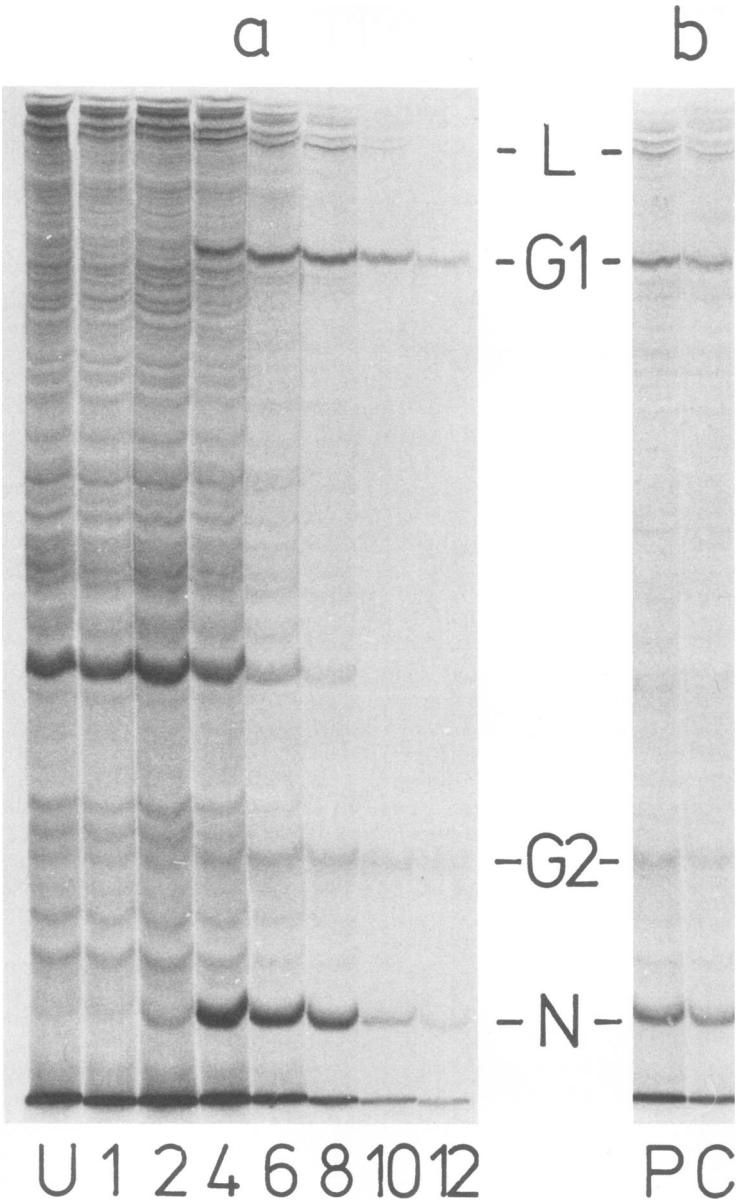

FIG. 1. *Autoradiogram showing polypeptide synthesis in infected cells. BSC-1 cells were infected at an input multiplicity of 40 PFU/cell. Cells were labeled for 1 h with [^{35}S]methionine (100,000 mCi/mmol; Radiochemical Centre, Amersham) at various times after infection. Before labeling, the cells were washed with Dulbecco phosphate-buffered saline. The label was then added in phosphate-buffered saline; in the pulse-chase experiment, the label was removed and the cells were washed and incubated further in Eagle medium containing 100 times the normal amount of methionine. After labeling, cells were scraped into 0.001 M Tris-hydrochloride, pH 9.0. Samples were then boiled for 2 min in 1% mercaptoethanol and 2% sodium dodecyl sulfate and subjected to electrophoresis in acrylamide gels using the discontinuous sodium dodecyl sulfate buffer system of Laemmli (5); gels were run and processed for autoradiography as previously described (9). (a) Time course, times at which labeling started are indicated (hours p.i.); (b) pulse-chase experiment: (U) uninfected cells; (P) 1-h pulse, starting at 8 h p.i.; (C) 1-h pulse as (P) followed by 3-h chase.*

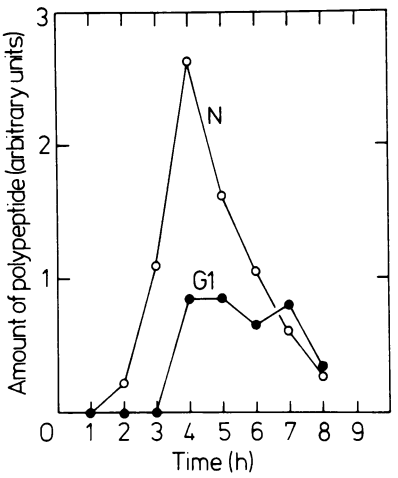

FIG. 2. *Kinetics of synthesis of polypeptides N and G1. Cells were labeled for 15 min with [^{35}S]methionine starting at the times indicated (hours p.i.). After electrophoresis and autoradiography, densitometer tracings were prepared with a Joyce-Loebl densitometer; the peaks were cut out and weighed. Infection, labeling, electrophoresis, and autoradiography were done as described in the legend to Fig. 1.*

maintained at a constant rate until 8 h p.i., when it declined. The onset of synthesis of polypeptides L and G2 was difficult to determine, as these polypeptides comigrated with host polypeptides, although it was clear that the rate of synthesis of polypeptide L was maximal at 4 h p.i., like that of polypeptide N. In some autoradiograms polypeptide L was detected as early as 3 h p.i., well before the onset of synthesis of polypeptide G1. The kinetics of synthesis of polypeptides G2 and G1 appeared to be similar; in many experiments, however, polypeptide G2 was not detected in autoradiograms. The reasons for this are not clear.

A decline in host protein synthesis commenced at about 5 h p.i.; by 7 h p.i. host synthesis was almost totally abolished. Virus-induced polypeptides continued to be made in relatively small amounts for many hours, the synthesis of all four polypeptides being still detectable at 22 h p.i. (results not shown). Examination of stained gels showed that only polypeptide N was made in sufficient amounts to be detected. This polypeptide was first visible as a stained band in gels of samples taken at 4 h p.i.; the intensity of the stained band reached a maximum in gels of samples taken at 7 h p.i., and no further increase in intensity was noted in gels of later samples (results not shown). Under the conditions used in these experiments, the production of infectious virus was first detected at about 7 h p.i. and continued until about 30 h p.i.

The possibility that the Bunyamwera virus-induced polypeptides were derived from a higher-molecular-weight precursor or precursors, as is the case with picornaviruses (3) and togaviruses (11), was investigated using several experimental approaches. The effect of amino acid analogs on polypeptide synthesis in infected cells was determined. It was assumed that incorporation of these analogs into the putative precursors would interfere with processing, leading to the appearance of these precursors in gel profiles. Accordingly, infected cells were treated for a short time with the amino acid analogs *p*-fluorphenylalanine and canavanine and were then labeled with [^{35}S]methionine in their presence. No high-molecular weight precursors were observed (results not shown). Labeling of infected cells for short periods (4 min) gave rise to the same polypeptide profile as longer labeling periods, no high-molecular-weight precursors being observed (Fig. 3). Pulse-chase experiments (1-h pulse followed by a 3-h chase) revealed that no changes in polypeptide mobility occurred during the chase period (Fig. 1). Finally, comparison of autoradiographs of virus-induced intracellular polypeptides (labeled for 15 min) and virion polypeptides G1 and N showed no significant mobility differences (Fig. 4).

Experiments with La Crosse virus have shown that the two intermediate-sized virion polypeptides G1 and G2 are glycosylated (7). Labeling of infected cells with [^{14}C]glucosamine showed that the analogous Bunyamwera virus-

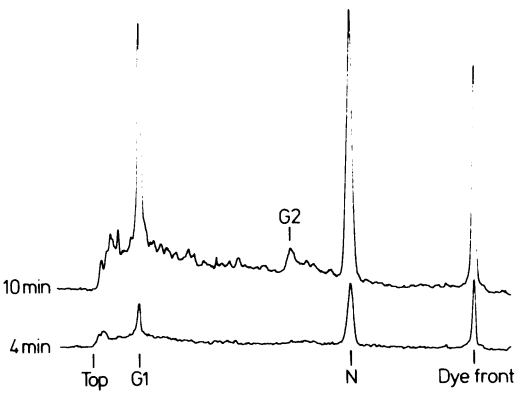

FIG. 3. *Densitometer tracing showing intracellular polypeptides detected after short labeling periods. Infected cells were labeled for the times indicated starting at 7 h p.i. After electrophoresis and autoradiography as described in the legend to Fig. 1, densitometer tracings were prepared with a Joyce-Loebl densitometer.*

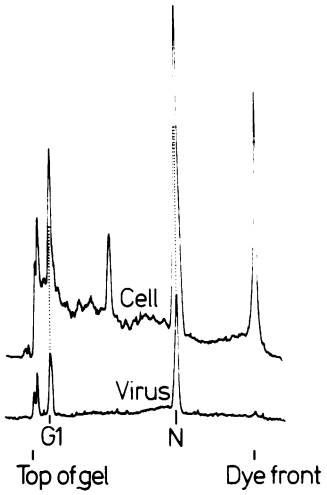

FIG. 4. *Densitometer tracings showing intracellular and virion-associated polypeptides G1 and N. Virus particles were prepared by labeling infected cell monolayers from 4 to 24 h p.i. with [^{35}S]methionine; the medium was then removed and centrifuged at 500 × g for 15 min, and the resulting supernatant was layered over a 20% sucrose cushion and centrifuged at 35,000 rpm for 2 h in a 50 Ti swing-out rotor. The pelleted virus particles were resuspended in a small volume. Infected cells were labeled for 15 min starting at 6 h p.i. Electrophoresis and autoradiography were done as described in the legend to Fig. 1. Polypeptide G2 was not resolved in this experiment.*

induced polypeptides were also glycosylated (results not shown).

Bunyamwera virus-particles have been shown to contain three genome segments (7). The demonstration of four virus-induced polypeptides coupled with our failure to find evidence of polypeptide processing by proteolytic cleavage suggests that there may be processing at the level of mRNA synthesis. We note that the molecular weights (7) of the three genome segments (2.9×10^6, 1.8×10^6, and 0.4×10^6) provide a coding capacity approximately 25% in excess of the total molecular weight of the four polypeptides. It is therefore possible that the virus could code for at least one polypeptide more than those resolved in the experiments reported here. Such a polypeptide could escape detection by comigration with another virus-induced polypeptide or by being synthesized in very small amounts.

A clear pattern of temporal control of virus-induced polypeptide synthesis has been shown in this study. This pattern of synthesis is unlike that reported for any other RNA virus, although temporal control of virus-induced polypeptide synthesis occurs during the growth cycle of influenza viruses (6, 12). It is of interest to note that biochemical and genetic evidence suggests that the Bunyaviruses, like the influenza viruses, possess segmented negative-strand RNA genomes (1, 2, 7, 8, 10).

LITERATURE CITED

1. **Clewley, J., J. Gentsch, and D. H. L. Bishop.** 1977. Three unique viral RNA species of snowshoe hare and La Crosse bunyaviruses. J. Virol. 22:459-468.
2. **Gentsch, J., and D. H. L. Bishop.** 1976. Recombination and complementation between temperature-sensitive mutants of a Bunyavirus, snowshoe hare virus. J. Virol. 20:351-354.
3. **Jacobson, M. F., J. Asso, and D. Baltimore.** 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. 49:657-669.
4. **Klenk, H. D., C. Scholtissek, and R. Rott.** 1972. Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology 49:723-734.
5. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
6. **Lamb, R. A., and P. W. Choppin.** 1976. Synthesis of influenza virus proteins in infected cells: translation of viral polypeptides, including three P polypeptides, from RNA produced by primary transcription. Virology 74:504-519.
7. **Obijeski, J. F., D. H. L. Bishop, F. A. Murphy, and E. L. Palmer.** 1976. Structural proteins of La Crosse virus. J. Virol. 19:985-997.
8. **Obijeski, J. F., D. H. L. Bishop, E. L. Palmer, and F. A. Murphy.** 1976. Segmented genome and nucleocapsid of La Crosse virus. J. Virol. 20:664-675.
9. **Pennington, T. H.** 1974. Vaccinia virus polypeptide synthesis: sequential appearance and stability of pre- and post-replicative polypeptides. J. Gen. Virol. 25:433-444.
10. **Porterfield, J. S., J. Casals, M. P. Chumakov, S. Ya. Gaidamovich, C. Hannoun, I. H. Holmes, M. C. Horzinek, M. Musagay, N. OkerBlom, and P. K. Russell.** 1975/76. Bunyaviruses and Bunyaviridae. Intervirology 6:13-24.
11. **Schlesinger, S., and M. J. Schlesinger.** 1972. Formation of Sindbis virus proteins: identification of a precursor for one of the envelope proteins. J. Virol. 10:925-932.
12. **Skehel, J. J.** 1973. Early polypeptide synthesis in influenza virus-infected cells. Virology 56:394-399.
13. **Smithburn, K. C., A. J. Haddow, and A. F. Mahaffy.** 1946. Neurotropic virus isolated from *Aedes* mosquitoes caught in Semliki forest. Am. J. Trop. Med. Hyg. 26:189-208.