SYNTHESIS IN VITRO OF VESICULAR STOMATITIS VIRUS PROTEINS IN CYTOPLASMIC EXTRACTS OF L CELLS

Hara P. Ghosh, Frances Toneguzzo and Steve Wells
Department of Biochemistry, McMaster University
Hamilton, Ontario, Canada.

Received July 20,1973

Summary: All five vesicular stomatitis virus (VSV) proteins, namely, L, G,N,NS and M are synthesized in vitro by a post-nuclear extract from cultured L cells infected with VSV. When, however, membrane bound polysomes are removed from the cytoplasmic extract only four virus specific proteins (L,N,NS and M) were synthesized.

Synthesis in vitro of virus specific protein in ribosomal systems from mammalian cells is currently intensively studied. Viral specific proteins can be synthesized in vitro by (i) the use of polysomes containing virus specific mRNA from infected cells or (ii) translation of viral mRNA by a ribosomal system which can be programmed by addition of exogenous mRNA. Thus using polysomal systems from infected cells, specific proteins for reovirus (1), adenovirus type 2 (2) and murine sarcoma-leukemia virus (3) were synthesized in vitro. RNA, isolated from picorna viruses like FMC, mengo, ME etc, and RNA tumor viruses like avian myeloblastosis, could be used to programme ribosomal systems from animal cells to synthesize virus specific proteins (4-6). Proteins specific to the double-stranded RNA containing reovirus have been synthesized by the cell-free translation of mRNA synthesized in vitro by the reovirus transcriptase complex (7,8). Recently the transcription product of vaccinia virus DNA has also been used to programme cell-free systems (9).

We have selected the single stranded RNA containing vesicular stomatitis virus (VSV) for studying virus-host cell interactions, in particular, the mechanism of regulation of protein synthesis during viral infection. VSV is a member of the rhabdovirus family (10) and the viral

RNA is complementary to the viral mRNA (11). The viral mPNA's are synthesized by the virus specific transcriptase complex containing VSV RNA (12-14) and in common with eukaryotic mRNA's (15) contain poly A residues (16, 17). Infection with VSV shuts down host protein synthesis and virus specific proteins, L(M.W. 190,000); G(M.W. 69,000); N(M.W. 50,000); NS(M.W. 40-45,000) and M(M.W. 29,000) are synthesized (18,19). Proteins L, N and NS are components of the nucleocapsid cores and the proteins G and M are structural proteins associated with the viral envelope (13,19,24).

In this communication we report the in vitro synthesis of VSV proteins using polysomes from L cells infected with VSV. All five proteins are synthesized by the unfractionated polysomes. When, however, membrane bound polysomes are removed the synthesis of the protein G is no longer observed.

Materials and Methods:

The growth of L cells, infection with VSV and isolation and characterization of VSV proteins have been described (14,18). L cells were infected with VSV (Indiana serotype HR-LT, obtained from Dr. L. Prevec) and the infected cells were harvested at 5-5 1/2 hours by centrifugation. The infected cells were disrupted according to Graziadei and Lengyel (7). The extract was centrifuged at 4,000 g. for 10 minutes and the supernatant fraction S-4 was used as the unfractionated polysomal system. Membrane bound polysomes were removed by further centrifugation at 25,000 g. for 20 minutes and the supernatant fraction S-25 was used as the free polysomal system. S-4 and S-25 were passed through Sephadex G-25 column before being stored in liquid N_2. The reaction mixture for protein synthesis contained: cell-free extracts 0.6 ml/ml, 40 mM Tris (pH 7.5), 100 mM KCl, 4 mM Mg acetate, 6 mM mercaptoethanol, 1 mM dithiothreitol, 1 mM ATP, 0.2 mM GTP, 0.6 mM CTP, 2 mg/ml creatine phosphate, 0.2 mg/ml creatine phosphokinase, 20 µM of each of 19 amino acids except the radioactive amino acid and [^{3}H] valine (sp. act. 29 Ci/mmole) 100 µci/ml or [^{35}S] methionine (sp. act. 10-60 Ci/

mmole) 50 μci/ml and was incubated at 37°. Aliquots were assayed as described earlier (20). The products were characterized by SDS-polyacrylamide gel electrophoresis. The reaction mixture was treated with 0.1 N NaOH at 37° for 15 minutes, neutralized, heated at 100° for 2 minutes in the presence of 2% SDS and 0.2% mercaptoethanol and finally dialyzed against the electrophoresis buffer containing 0.1% SDS (7). Electrophoresis was performed according to Kang and Prevec (18). Each sample also contained an internal marker of viral proteins labelled with ^{35}S or ^{3}H. The gels were fractionated by a commercial gel fractionator (Gilson Electronics, Wis.) (21) and counted for ^{3}H and ^{35}S in a xylene-triton based scintillation fluid (22).

Results and Discussion:

The polysomal systems obtained from infected or uninfected L cells synthesized proteins very actively. Figure 1 shows the incorporation of [^{3}H] val and [^{35}S] met by the S-4 extract obtained from VSV-infected L cells. The incorporation increases rapidly for the first 20 minutes and then at a much slower rate up to 90 minutes. The addition of a 100-fold excess of non-radioactive methionine at different intervals of time starting at 5 minutes prevents further incorporation of ^{35}S met. The amount of [^{35}S] met incorporated, however, remains constant. An essentially similar time course of incorporation is observed with the S-4 system from uninfected and S-25 system from infected or uninfected cells. The incorporation by extracts from uninfected cells is about 1.5-fold higher than that from infected cell extracts. The incorporation of radioactive amino acids is sensitive to puromycin and cycloheximide. The addition of the bacterial and mitochondrial protein synthesis inhibitor chloramphenicol has no effect on the incorporation.

The polypeptides synthesized in vitro are characterized by their electrophoretic mobilities on a SDS-polyacrylamide gel in comparison with an internal marker of radioactive VSV proteins. It is observed that the polypeptides synthesized by a S-4 extract from uninfected L cells are a

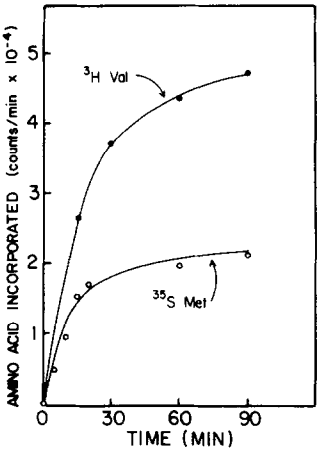

Fig. 1. Time course of incorporation of [^{3}H] val or [^{35}S] met by S-4 extract from VSV infected L cells. 5×10^8 L cells were infected with VSV at a m.o.i. of 20 for 5.5 hrs. S-4 extract prepared from infected cells was used and 0.01 ml aliquots were counted for hot trichloroacetic acid insoluble materials.

heterogeneous population which do not move with the marker viral proteins (Fig. 2A). A similar pattern is also obtained with S-25 extract from uninfected cells. In contrast the S-4 extracts from infected cells synthesize polypeptides which move with the marker viral proteins (Fig. 2B). Host polypeptide synthesis appears to be inhibited and five proteins corresponding to VSV proteins, L,G,N,NS and M are synthesized.

Electrophoresis of the polypeptides synthesized by S-25 extract from infected cells reveals the absence of the polypeptide band moving with marker G protein (Fig. 2C). The four other proteins again move with authentic viral proteins. Thus the polypeptide which has the same mobility as G protein appears not to be synthesized in absence of membrane bound polysomes. It has been suggested that the two structural proteins, G and M may be synthesized by membrane bound polysomes (23). Alternatively, the processing of synthesized proteins into G protein may need the presence of membrane.

Identical results were also obtained when [^{35}S] met or [^{3}H] amino acid mixture was used.

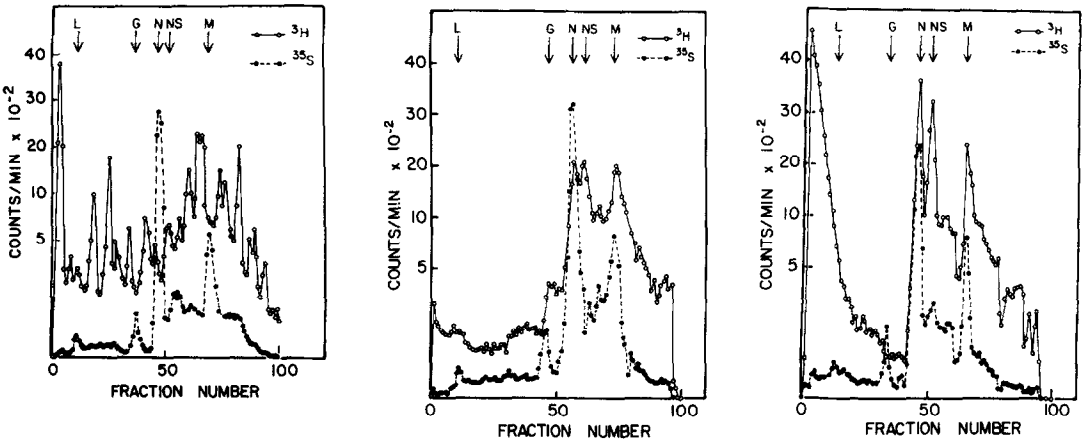

Fig. 2. Electrophoretic profiles of [^{3}H] val labelled proteins synthesized in vitro by cell-free extracts from L cells. [^{35}S] met labelled VSV proteins were used for internal calibration. [^{3}H] val labelled proteins synthesized (approximately 70,000 cpm) were isolated as described in text, mixed with [^{35}S] met labelled VSV proteins (approximately 25,000 cpm) and electrophoresed on a 10 cm gel containing 7.5% acrylamide and 0.1% SDS. The gel was fractionated into 1 mm sections and counted for ^{3}H and ^{35}S.
A (Left). S-4 extract from uninfected L cells.
B (Centre). S-4 extract from VSV infected L cells.
C (Right). S-25 extract from VSV infected L cells.

The results also show that a protein having same electrophoretic mobilility as NS protein is made in significant amount by the extracts. The protein NS is present in the virus as a minor component as part of the nucleocapsid cores (13,24).but is present in large amounts in the infected cell (18,19). Synthesis in vitro of large amounts of NS proteins by polysomes from infected cells thus suggests that NS protein may have some role in the VSV infected cell.

It may be pointed out that a minor radioactive peak appearing between proteins NS and M is present in both the in vitro synthesized product and in the marker viral proteins. The significance of this additional peak is not clear at the present time.

Definitive identification of the products of cell-free system as VSV proteins will require further studies involving finger printing and immunological identification.

Acknowledgement: We like to thank Medical Research Council of Canada for financial support (Grant MT 3514). We also express our sincere gratitude to Dr. L. Prevec, Department of Biology, McMaster University, for his kind help and guidance and also allowing us to use his facilities for cell culture. We also thank Dr. P.K. Sircar for assistance. H.P.G. is a Medical Research Council of Canada Scholar. S.W. and F.T. are undergraduate research trainees.

References:

(1) M.J. McDowell and W.K. Joklik, Virology, 45, 724 (1971).
(2) H. Caffier and M. Green, Virology, 46, 98 (1971).
(3) G. Shanmugam, G. Vecchio, D. Attardi and M. Green, J. Virol., 10, 447 (1972).
(4) I.M. Kerr and E.M. Martin, J. Virol., 7, 438 (1971).
(5) K.L. Eggen and A. Shatkin, J. Virol., 9, 636 (1972).
(6) J.G. Vidrine, C.S. Wang and R.B. Arlinghaus, Biochem. Biophys. Res. Commun., 46, 538 (1972).
(7) W.D. Graziadei III and P. Lengyel, Biochem. Biophys. Res. Commun., 46, 1816 (1972).
(8) M.J. McDowell, W.K. Joklik, L. Villa-Komaroff and H.F. Lodish, Proc. Nat. Acad. Sci., 69, 2649 (1972).
(9) F. Fournier, D.R. Tovell, M. Esteban, D.A. Metz, L.A. Ball and I.M. Kerr, FEBS letters, 30, 268 (1973).
(10) A.F. Howatson, Adv. Virus Res., 16, 165 (1970).
(11) A.S. Huang, D. Baltimore and M. Stampfer, Virology, 42, 946 (1970).
(12) D. Baltimore, A.S. Huang and M. Stampfer, Proc. Nat. Acad. Sci., 66, 572 (1970).
(13) D.H.L. Bishop and P. Roy, J. Virol., 10, 234 (1972).
(14) H. Galet, J.G. Shedlarski and L. Prevec, Can. J. Biochem., 51, 721 (1973).
(15) M. Adesnik, M. Salditt, W. Thomas and J.E. Darnell, J. Mol. Biol., 71, 21 (1972).
(16) E. Ehrenfeld and D.F. Summers, J. Virol., 10, 683 (1972).
(17) H. Galet and L. Prevec, Nature New Biol., 243, 200 (1973).
(18) C.Y. Kang and L. Prevec, Virology, 46, 678 (1971).
(19) R.R. Wagner, L. Prevec, F. Brown, D.F. Summers, F. Sokol and R. MacLeod, J. Virol., 10, 1228 (1972).
(20) K. Ghosh and H.P. Ghosh, J. Biol. Chem., 247, 3369 (1972).
(21) K.C. Medappa, C. McLean and R.R. Rueckert, Virology, 44, 259 (1971).
(22) L.E. Anderson and W.O. McClure, Anal. Biochem., 51, 173 (1973).
(23) R.R. Wagner, M.P. Kiley, R.M. Snyder and C.A. Schaitman, J. Virol., 9, 672 (1972).
(24) S.U. Emerson and R.R. Wagner, J. Virol., 10, 297 (1972).