Selective Suppression of Cellular Protein Synthesis in BHK-21 Cells Infected with Rabies Virus

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Under hypertonic conditions, cellular protein synthesis is selectively suppressed in rabies virus-infected cells. The resistance of viral polypeptide synthesis to hypertonic conditions provides a means to study intracellular viral protein synthesis and may represent a property common to translation of many viruses.

Saborio et al. (12) reported that peptide chain initiation is suppressed in uninfected and in poliovirus-infected HeLa cells exposed to hypertonic (high NaCl) conditions. England et al. (4a) observed a selective suppression of cellular protein synthesis under hypertonic conditions in African green monkey kidney (AGMK) cells infected with simian virus 40 (SV40). Because cellular protein synthesis is not normally inhibited in SV40-infected AGMK cells, the high salt technique facilitated the analysis of SV40 polyprotein synthesis. This report describes a similar selective suppression of cellular protein synthesis under hypertonic conditions in BHK-21 cells infected with rabies virus, a rhabdovirus. After the present work was completed, we became aware that Nuss et al. (10) have independently found that hypertonic conditions selectively suppressed host protein synthesis in poliovirus-, vesicular stomatitis virus- and reovirus-infected cells relative to the synthesis of host polypeptides. This report confirms and extends the observations made by Nuss et al. (10). These observations suggested that a significant difference in the mechanism of viral and cellular protein synthesis may exist.

Under normal conditions, protein synthesis in BHK-21 cells is not significantly inhibited by rabies virus until several days after infection (9; H. P. Madore and J. M. England, unpublished observation). Exposure of the infected cells to medium containing increasing concentrations of NaCl resulted in a decrease of [3H]amino acid incorporation into total acid-precipitable protein (inset, Fig. 1). Sodium dodecyl sulfate-polyacrylamide gel analysis of the labeled polypeptides in the cytoplasmic fractions of infected cells showed a nonuniform decrease in the labeling of various polypeptide size classes. The polypeptides that coelectrophoresed with [14C]amino acid-labeled viral polypeptides (G, N, M1, M4) (15) showed the least amount of suppression. Defining arbitrarily as viral the polypeptides that coelectrophoresed with [14C]amino acid-labeled viral polypeptides and as cellular the polypeptides in fractions 10 to 20 where no viral structural polypeptides could be detected, we observed an enhancement of [3H]amino acid incorporation into the viral relative to the representative cellular polypeptides (fractions 10 to 20) as hypertonicity increased. Between 290 and 602 mOsM, significant differences of [3H]amino acid incorporation among the viral polypeptides could not be detected. Because the amino acid pools for viral and cellular protein synthesis should be equally affected by hypertonic conditions, the relative enhancement of incorporation into viral versus cellular polypeptides most likely reflects a relative enhancement of viral polypeptide synthesis. Under these experimental conditions, i.e., 15 min of preincubation in hypertonic medium, an enhancement of ninefold was obtained at 553 mOsM. This level of enhancement of viral protein synthesis is similar to that obtained with the SV40-AGMK system (4a).

We next compared mock-infected and rabies virus-infected cells labeled under identical osmotic conditions to determine whether the labeled polypeptides coelectrophoresing with virus polypeptides truly represented cell-associated viral polypeptides and not cellular polypeptides resistant to hypertonic conditions. The polypeptide patterns in the cytoplasmic fractions of mock-infected and infected cells labeled under isotonic (290 mOsM) conditions were found to be almost identical (Fig. 2A). In contrast, the cytoplasmic fraction of infected cells labeled under hypertonic (553 mOsM)
FIG. 1. Effect of hypertonic conditions on protein synthesis in rabies virus-infected BHK-21 cells. Confluent monolayers (2.0 × 10^7 cells/100-mm dish) of BHK-21 cells were infected with 50 PFU/cell of rabies virus (ERA strain). After 30 min at 37 C, unadsorbed virus was removed, and cells were washed two times with PBS and then incubated for 20 h in 10% fetal calf minimal essential medium at 37 C in a 5% CO_2 atmosphere. Separate cultures were labeled with [3H]amino acid mixture (20 μCi/ml; average specific activity, 4.1 Ci/mmol) (Schwarz/Mann mixture no. 3130-08, Schwarz-Bio Research, Orangeburg, N.Y.) for 1 h at 37 C under isotonic (280 mosM) or hypertonic conditions (418, 516, 553 602, 656 mosM) by the following procedure. Each culture was incubated with Earle salts plus 10% fetal calf serum at 37 C for 15 min, then in Earle salts plus 10% fetal calf serum containing the appropriate amount of excess NaCl for 15 min, and then in media of similar tonicity containing [3H]amino acid mixture for 1 h. The toxicity of the media was determined with an automatic osmometer (Osmette-A, Precision Systems, Inc., Sudbury, Mass.). At the end of the pulse, each culture was washed two times with ice-cold NT buffer (0.13 NaCl, 0.05 M Tris-hydrochloride, pH 7.8), scraped off the plate with 1 ml of Tris-K-Mg buffer (0.01 M KCl, 0.0015 M MgCl₂, 0.01 M Tris-hydrochloride, pH 6.7), and then homogenized with a Dounce homogenizer. The crude nuclear fraction was pelleted at 250 g, washed once with Tris-K-Mg buffer, the cytoplasmic fractions were pooled, and the samples were frozen at −70 C. Total protein was determined by a modified Lowry procedure (11), and total acid-precipitable radioactivity was determined by standard procedures. Protein (400 μg) of the cytoplasmic fractions and a small amount of purified [14C]amino acid-labeled rabies virus (13) were prepared for electrophoresis by the procedure of Sokol et al. (14). The samples were electrophoresed on 7.5% polyacrylamide gels in 0.1% sodium dodecyl sulfate-0.1% PO₄ buffer (pH 7.2) as 3.5 mA/gel for 16 h (14). The gels were fixed, cut into 1-mm slices, and then incubated overnight at 37 C in 8 ml of a 5% Protosol-liquid scintillation fluid mixture. The position of the viral marker polypeptides is indicated for each gel. For panels (C) and (D), the position of the viral polypeptides was determined by parallel electrophoresis of the [14C]amino acid-labeled rabies virus. The molecular weights of the viral polypeptides G, N, M₁, and M₂ are 80,000, 62,000, 40,000, and 25,000, respectively (13). (Inset) Trichloroacetic acid-precipitable radioactivity incorporated per microgram of total cellular protein as a function of increasing hypertonicity. Arrows indicate the samples analyzed on polyacrylamide gels.
Fig. 2. Comparison of protein synthesis in rabies virus-infected and mock-infected BHK-21 cells under hypertonic conditions. Confluent cultures of BHK-21 cells (5 x 10⁶/60-mm dish) were either infected as described in the legend to Fig. 1 with 50 PFU/cell of rabies virus (ERA strain) or mock-infected with 0.2% bovine serum albumin-minimal essential medium and then incubated for 24 h at 37 C in a 5% CO₂ atmosphere. Each set of cultures was then labeled for 1 h with a [³H]amino acid mixture (40 μCi/ml, 4.1 Ci/mmol) under isotonic (290 mOsM) or hypertonic (553 mOsM) conditions as detailed in the legend to Fig. 1. The cells were divided into crude nuclear and cytoplasmic fractions and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in the legend to Fig. 1. Symbols: ○, rabies-infected BHK-21 cells; O, mock-infected BHK-21 cells.

conditions showed four distinct peaks that coelectrophoresed with the rabies virus polypeptides (Fig. 2C), whereas the cytoplasmic fractions of the mock-infected cells labeled under similar conditions contained no polypeptides that coelectrophoresed with the rabies virus polypeptides (Fig. 2C). These results indicate that the polypeptides coelectrophoresing with viral polypeptides in infected cells labeled under hypertonic conditions are cell-associated viral polypeptides. In addition, a minor polypeptide migrating to the left of the glycoprotein (G) in the cytoplasm of infected cells labeled under hypertonic conditions was not present in the mock-infected cells under similar conditions. The origin of the polypeptide, viral or cellular, remains to be determined.

The crude nuclear fractions of the infected cells labeled under hypertonic conditions also contained polypeptides that coelectrophoresed with rabies virus polypeptides, whereas the mock-infected cells labeled under similar conditions did not (Fig. 2B and D). Since rabies virus proteins have previously been found only in the cytoplasm (1-9), these polypeptides are most likely associated with cytoplasmic fragments that contaminate the nuclear fraction. The relative proportion of viral polypeptides in the nuclear fraction was different from that observed in the cytoplasmic fraction (Fig. 2B and
D). This different ratio is not an artifact of increasing NaCl concentration (Fig. 1; nuclear fractions not shown), but may represent different cytoplasmic sites in the cell for the synthesis or processing of the polypeptides.

The polypeptide synthesis of SV40 and of rabies virus is more resistant to hypertonic conditions than cellular polypeptide synthesis. Nuss et al. (10), in an independent study, have shown that polyoma virus, vesicular stomatitis virus, and reovirus polypeptide synthesis are also resistant to hypertonic conditions. The manifestation of this phenomenon with several distinct groups of viruses lends support to the hypothesis that a fundamental difference may exist between viral and cellular polypeptide chain initiation, possibly due to a basic difference in viral and cellular mRNA. The use of hypertonic conditions also provides a valuable tool to study protein synthesis of viruses, like rabies, that do not significantly inhibit host protein synthesis during the course of infection.

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