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NOTES

Differential Inhibition of Vesicular Stomatitis Virus Polypeptide Synthesis by Hypertonic Initiation Block

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Synthesis of the vesicular stomatitis virus membrane matrix protein and the glycoprotein is inhibited to a greater extent than the synthesis of the nucleocapsid protein, the nonstructural protein, and the large protein when the rate of peptide chain initiation is reduced by exposure of vesicular stomatitis virus-infected cells to hypertonic medium. It is concluded that the relative sensitivity of individual viral polypeptide synthesis to hypertonic initiation block is independent of the site of synthesis, i.e., whether on membrane-associated or free polyribosomes.

It was previously reported that brief exposure of cultured animal cells to excess NaCl in the growth medium results in selective and reversible inhibition of polypeptide chain initiation, whereas polypeptide chain elongation, termination, and post-translational processing proceed unaffected (15). Furthermore, when RNA virus-infected cells are exposed to suitable hypertonic conditions, translation of host mRNA is selectively inhibited (14). Moreover, it has recently been demonstrated that, when the rate of polypeptide chain initiation in mouse plasmacytoma cell cultures is reduced by exposure to hypertonic medium, the synthesis of the immunoglobulin G (IgG) polypeptides, particularly that of the light chain, is considerably less affected than the synthesis of non-IgG polypeptides (Nuss and Koch, submitted for publication). These results indicate that the efficiency with which translation is initiated (the intrinsic translational efficiency) is greater for viral mRNA and mRNA coding for the IgG light- and heavy-chain polypeptides than for other cellular mRNA. A model recently proposed for the regulation of α- and β-globin mRNA translation (10) provides a theoretical basis consistent with this interpretation.

Since myeloma IgG synthesis is thought to occur only on membrane-associated polysomes (4), it was considered important to determine whether exposure of cells to hypertonic medium results in a preferential inhibition of protein synthesis on either membrane-associated or free polysomes. Vesicular stomatitis virus (VSV)-infected cells provide a convenient system to approach this question, since only one of the five VSV polypeptides, the glycoprotein, is synthesized on membrane-bound polysomes (3, 7, 8).

Although viral polypeptide synthesis is reduced only slightly by hypertonic conditions, which almost completely inhibit host polypeptide synthesis (14), still higher concentrations of NaCl result in the progressive inhibition of VSV-specific polypeptide synthesis. Figure 1 shows the results of analysis by polyacrylamide gel electrophoresis of cytoplasmic extracts of VSV-infected cells, pulse labeled for 15 min at 4 h postinfection, after a 15-min preincubation in isotonic (110 mM NaCl) or hypertonic (250 mM NaCl) medium. Total radioactive amino acid incorporation was inhibited 75% by this level of excess NaCl. The profiles are normalized to allow a comparison of the distribution of radioactive precursors into polypeptides under the two conditions. In such a comparison, an increase in the relative incorporation into a particular protein indicates that the synthesis of that polypeptide is reduced to a lesser extent than the average inhibition of protein synthesis. A decrease in the relative incorporation indicates that the synthesis of that polypeptide is inhibited to a greater extent than the average. Although exposure of VSV-infected cells to hypertonic medium results in a reduction in the incorporation of radioactive precursors into all viral polypeptides, it is clear from Fig. 1 that the relative incorporation into the membrane

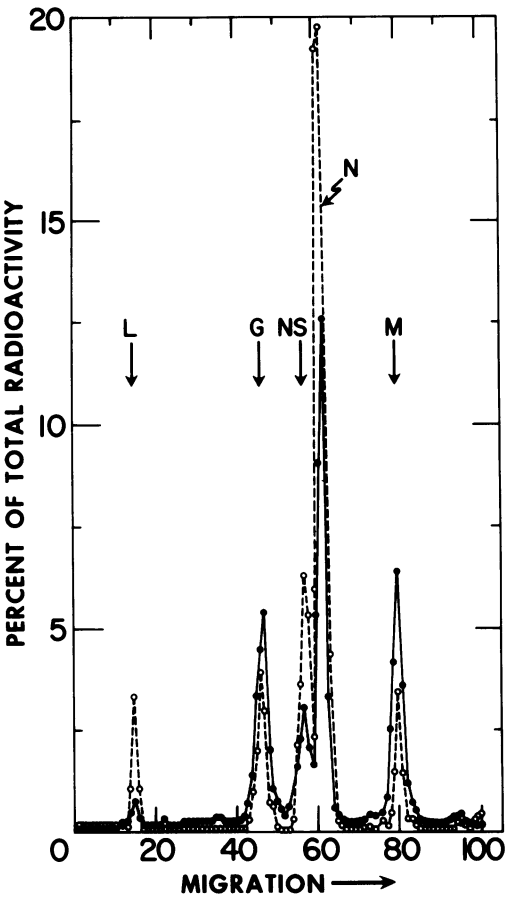

FIG. 1. *Analysis by polyacrylamide gel electrophoresis of cytoplasmic extracts from cells pulse labeled in isotonic (●) and hypertonic (250 mM NaCl; ○) medium. Adsorption of VSV, serotype Indiana (12), was allowed at room temperature for 20 min with HeLa S_3 cells suspended at a density of 10^7 cells/ml in serum-free, Joklik-modified minimal essential medium (MEM) (GIBCO F-13). The multiplicity of infection was 10 PFU/cell. After adsorption, the cell suspensions were diluted to a density of 10^6 cells/ml with MEM containing 5% fetal calf serum. After the appropriate time of incubation at 37 C, aliquots of infected cells were harvested by centrifugation and resuspended at a density of 4×10^6 cells/ml in 3.8 ml of MEM–25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4, containing 1/20 the normal complement of methionine and leucine. The NaCl concentration of the MEM medium, normally 110 mM, was adjusted, and after a 15-min incubation at 37 C the cells were pulse labeled with 25 μCi of [^{35}S]methionine and 50 μCi of [^3H]leucine for 15 min. After removal of a 50-μl aliquot for the determination of radioactive precursor incorporated into protein (11), each sample was diluted with 10 volumes of semifrozen, serum-free MEM containing a large excess of unlabeled methionine and leucine. The cells were washed once with and resuspended in the same medium. After a 15-min incubation period at 37 C to chase nascent peptides to completion, cytoplasmic extracts were prepared as previously described (14). Extracts were analyzed on 10-cm, 10% polyacrylamide-sodium dodecyl sulfate gels according to Laemmli (9). It must be stressed that the pre-exposure of cells to hypertonic conditions is necessary to allow preinitiated ribosomes to complete translation of those mRNA molecules on which initiation has been blocked; 15 min is more than sufficient for this process (15).*

matrix protein (M) and glycoprotein (G) is markedly reduced, whereas the relative incorporation into the nucleocapsid (N), nonstructural (NS), and large (L) proteins is substantially increased.

The NaCl concentration-dependent change in the relative synthesis of the five polypeptides is presented in Fig. 2 as the percentage of total incorporation into virus-specific proteins for each polypeptide. The percentage of total incorporation into the N, NS, and L polypeptides increases from 38.7 to 55.8%, 13.1 to 19.3%, and 2.3 to 5.8%, respectively, over the NaCl concentration range used. The values for the M and G

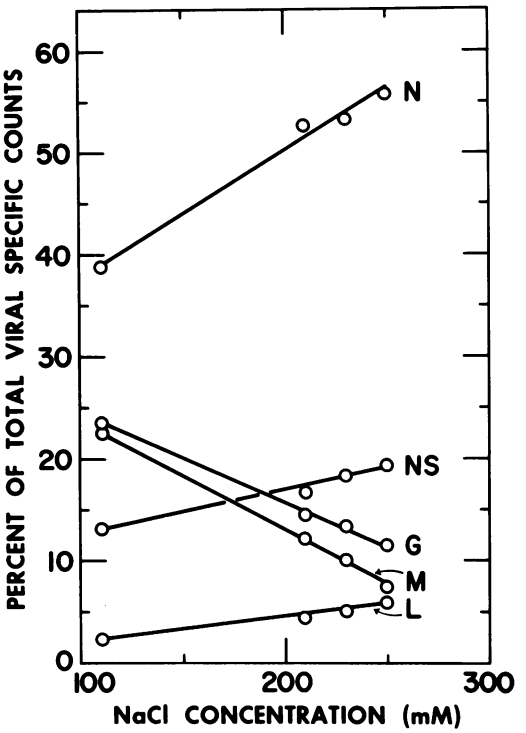

FIG. 2. *NaCl concentration-dependent change in relative synthesis of each VSV polypeptide presented as the percentage of total [^{35}S]methionine + [^{3}H]leucine incorporation into viral-specific polypeptides. The concentrations of NaCl were 210, 230, and 250 mM.*

proteins decrease from 22.4 to 7.3% and 23.4 to 11.5%, respectively. When even higher concentrations of excess NaCl are used, the synthesis of the N, NS, and L proteins continues in the apparent absence of M and G synthesis. However, since the G and M proteins are synthesized on membrane-associated and free polysomes, respectively, we conclude that there is no correlation between the site of synthesis of individual viral polypeptides and the relative sensitivities of the synthesis of these polypeptides to hypertonic initiation block.

The possibility that during the 15-min pulse labeling hypertonic conditions may affect the turnover or excretion of the M and G polypeptides preferentially has not been ruled out. However, it has been demonstrated in mouse plasmacytoma cultures that excess NaCl in the growth medium does not affect the turnover or secretion of the Ig light or heavy chains, which are also both membrane-associated polypeptides (Nuss and Koch, submitted for publication).

We previously suggested that, in addition to competition between host and viral mRNA for binding of ribosomes, a major event in the suppression of host translation directed by poliovirus in HeLa cells and VSV in mouse plasmacytoma cells is a reduction in the overall rate of polypeptide chain initiation, and that under these conditions the translation of viral mRNA proceeds, by virtue of its higher intrinsic translational efficiency, whereas the translation of host mRNA is substantially reduced (14; Nuss and Koch, submitted for publication). Furthermore, we predicted that the synthesis of those polypeptides, which showed resistance to hypertonic initiation block, would also show resistance to suppression following virus infection. This prediction has recently been confirmed in studies with VSV-infected plasmacytoma cells, which show that IgG protein synthesis is more resistant than non-IgG polypeptide synthesis to both hypertonic initiation block and suppression following virus infection (Nuss and Koch, submitted for publication). It is, therefore, of particular interest that in VSV-infected HeLa cells superinfected with poliovirus a preferential inhibition of the VSV G and M polypeptides is observed (6). Thus, hypertonic initiation block and poliovirus superinfection both preferentially inhibit the synthesis of the VSV G and M protein relative to the synthesis of the N, NS, and L polypeptides. It was previously shown that the translation of poliovirus mRNA in poliovirus-infected HeLa cells is more resistant to hypertonic initiation block than is the translation of VSV mRNA in VSV-infected HeLa cells (14).

It is difficult, at present, to correlate the results presented in this communication with the mechanisms of regulation of VSV polypeptide synthesis in infected cells. However, studies now in progress comparing the effect of inhibitors of polypeptide chain elongation and hypertonic initiation block on the synthesis of VSV polypeptides at various times during the infectious cycle may provide some insights into this mechanism.

The process of protein synthesis is complex. It is unclear to what extent the structure of mRNA, ribosome conformation, and the activity of initiation and/or interfering factors determine the relative efficiency with which the translation of different mRNA's is initiated. Recent work has provided new information concerning the nature and structure of eukaryotic viral mRNA-ribosome binding sites (1, 2, 5, 13). A study comparing the primary structure of the ribosome binding site of well-defined eukaryotic viral or cellular mRNA, e.g., the VSV

mRNA's, with the relative sensitivities of the translation of these messengers to inhibition by hypertonic initiation block should provide fundamental information concerning this important question.

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