Regulation of Translation of Eukaryotic Virus mRNAs

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A. Introduction

One of the best studied examples of the regulation of translation is the inhibition of host cell protein synthesis following viral infection (Bablanian, 1975; Smith and Carrasco, 1978). In several virus/host systems the bulk of viral proteins are synthesized under conditions in which the initiation of translation of cellular mRNAs is strongly inhibited.

We have raised the possibility that changes in the concentration of ions in the cellular cytoplasm following viral infection could specifically block cellular protein synthesis. We showed for picornavirus infected cells that the cellular membrane of the host becomes leaky at the time viral protein synthesis begins (Farham and Epstein, 1963; Carrasco, 1978). Moreover, optimal translation of picornavirus RNA in vitro occurs under ionic conditions in which the synthesis of cellular proteins is strongly restricted (Carrasco and Smith, 1976). In the present work, we show that the higher optimum of monovalent ions required for in vitro translation is also observed with mRNAs from papovaviruses, togaviruses, rhabdoviruses and myxoviruses. These observations are discussed in the general context of viral development, the shut-off of host protein synthesis and as a possible explanation for the differential inhibition of viral and cellular mRNA translation following exposure of cells to hypertonic medium (see Koch et al. in this volume).

B. Results

1. Papovaviruses

The in vitro translation of the mRNAs present in the cytoplasm of polyoma infected 3T6 cells is illustrated in Fig. 1. The monovalent ion optimum for the synthesis of actin (a cellular protein) and VP1 (a viral protein) differ by 30 mM K^+, indicating that the polyoma 16S mRNA is more efficiently translated in vitro at higher potassium concentrations. This result was obtained using either the chloride or acetate salt of potassium. These results indicate: a) that in vitro changes in the concentration of monovalent ions do not produce an indiscriminate inhibition on the translation of all mRNAs; ions have to be regarded as specific effectors of protein synthesis, because they inhibit the translation of some mRNAs and at the same time stimulate translation of

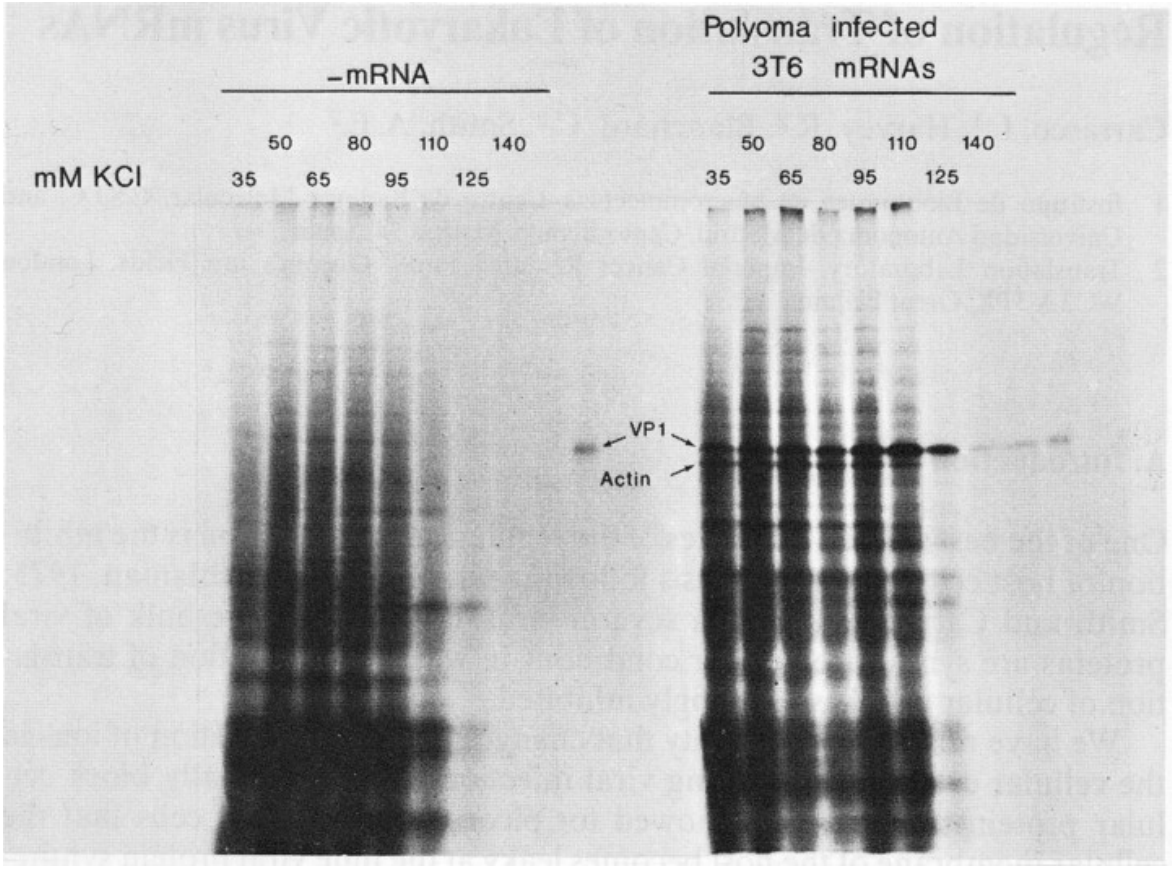

Fig. 1. Analysis by polyacrylamide gel electrophoresis of the products synthesized in the wheat germ system in response to mRNAs from 3T6 cells infected with polyoma virus. Effect of the KCl concentration

others and b) that from the relative synthesis of two proteins in vitro we cannot deduce directly the actual proportion of their mRNAs in the cell-free system.

It has been suggested that the difference in monovalent cation requirement between picornavirus and host cell mRNAs reflects the difference in the size of the mRNAs. If this hypothesis were correct, it would follow that those mRNAs requiring a high concentration of monovalent cations for optimal translation are large and code for high molecular weight proteins. The present results do not support this conclusion, because the synthesis of VPl has a higher monovalent ion requirement as compared to the synthesis of actin, yet both proteins have a similar molecular weight and similar sized mRNAs (see also the results obtained in section B 2).

In vitro changes in the concentration of divalent cations (magnesium or calcium) did not produce a differential effect on the synthesis of VPl and actin. This finding indicates to us that the changes of monovalent ions are more likely to be involved in the shut-off of host protein synthesis than divalent cations.

2. Togaviruses

Semliki Forest virus has a 42S genomic RNA which is translated early during infection to produce non structural proteins. Later, coat proteins are synthes-

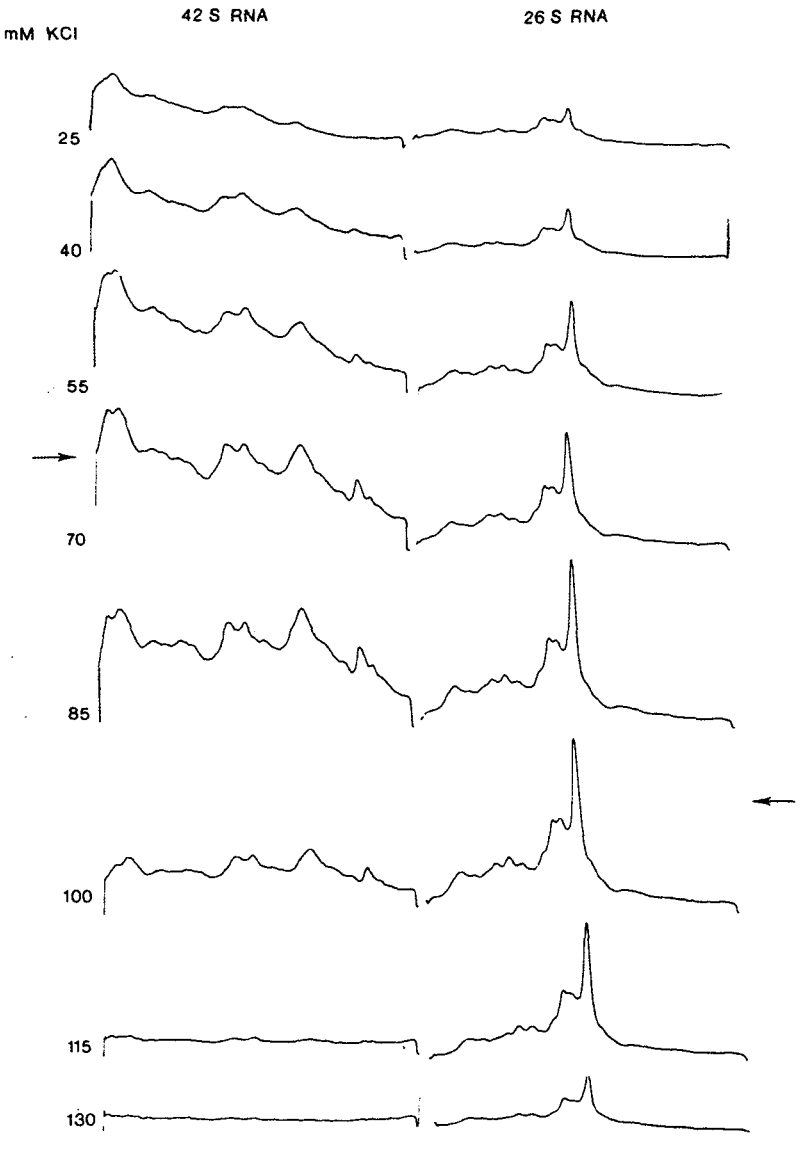

Fig. 2. Effect of the KCl concentration on the in vitro translation of Semliki Forest Virus 42S and 26S mRNAs. The arrows in the scans indicate the optimum for translation

ized from the subgenomic 26S mRNA, and at the same time, the synthesis of host and viral non structural proteins are inhibited in parallel (Lachmi and Kaariainen, 1977). This system provided us with a good model to test our hypothesis. Since the viral 26S mRNA is translated when shut-off occurs, the model predicts it should have a high optimum of monovalent ions for translation. On the other hand the viral 42S mRNA which is translated before host protein synthesis is inhibited should have a lower optimum of monovalent ion concentration for in vitro translation similar to that of host cell mRNA. Fig. 2 shows that indeed this is the case. Under the ionic conditions in which maximal synthesis of coat proteins occurs the translation of the genomic 42S mRNA is severely inhibited.

3. Rhabdoviruses and Myxoviruses

Vesicular stomatitis virus (VSV), a rhabdovirus, is probably the best studied species in class V viruses (Smith and Carrasco, 1978). It produces a drastic inhibition of cellular protein synthesis after infection (Wertz and Youngner, 1972). A similar inhibitory effect on translation is observed when susceptible cells are infected by influenza virus, a myxovirus. The in vitro translation of the mRNAs from these two viruses is shown in Fig. 3. Again, the KCl optimum for the in vitro translation of influenza and VSV mRNAs is higher than the optimum required to translate cellular mRNAs.

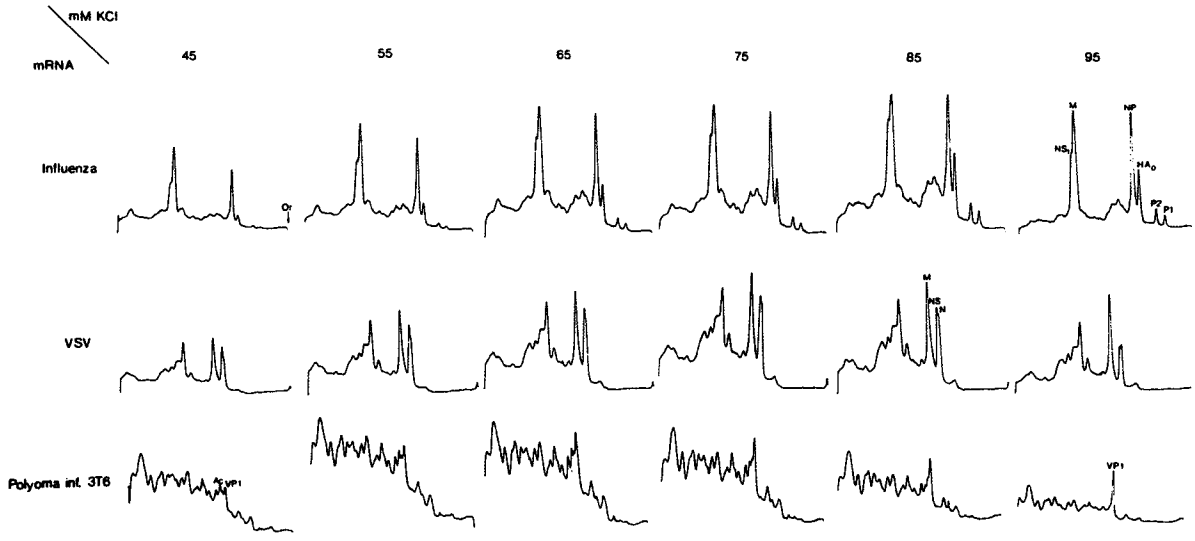

Fig. 3. Effect of the KCl concentration on the in vitro translation of VSV and influenza mRNAs. The products synthesized in the wheat germ system were analysed by PGE and the autoradiogram was analysed in a densitometer

C. Discussion

Several theories have been advanced to explain the regulation of translation in eukaryotic organisms (Lodish, 1976). Nowadays the existence of initiation factors specific for different mRNAs is considered unlikely. Recently evidence has accumulated which indicates that the phosphorylation of initiation factors and ribosomal proteins occurs in vitro. However, as yet there is little direct evidence to show that these modifications are responsible for the in vivo regulation of translation, observed under different physiological conditions.

It is well known that ions are involved in the regulation of a great many biological processes and metabolic reactions. The results shown in this work indicate that changes in monovalent ions in vitro are able to produce effects that mimic those observed in vivo after viral infection. In addition, we also know that the membrane of many viral infected cells becomes leaky to ions and small metabolites at the time as the bulk of viral proteins are synthetized (Carrasco, 1978; Contreras and Carrasco, manuscript in preparation). These

results also suggest that the effects of hypertonic medium in some virus infected cells can be explained in a very simple way: hypertonic medium produces a higher concentration of monovalent ions in the cellular cytoplasm and thus favours the translation of some viral mRNAs, and inhibits the translation of other cellular mRNAs. Whether changes in salt concentration are directly involved in shut-off, or whether other effects such as competition between mRNAs or distruction of specific initiation factors play a role, remains to be established.

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