Hypothesis

THE INHIBITION OF CELL FUNCTIONS AFTER VIRAL INFECTION

A proposed general mechanism

Luis CARRASCO*
Department of Molecular Virology, Imperial Cancer Research Fund, London WC2A 3PX, England

Received 14 February 1977

1. Introduction

The development of a virus in its host cell often involves interference with cellular functions [1–4]. One of the best-studied processes inhibited after viral infection is host-cell protein synthesis (the shut-off phenomenon). Since it was first described for picornavirus [5–7] several observations have been firmly established:

(1) Total protein synthesis decreases after infection, in such a way that the bulk of synthesis of viral proteins occurs in conditions in which cellular protein synthesis is drastically inhibited [1–4].

(2) In the absence of virus replication shut-off still occurs [8]. This infers that virus particles themselves are enough to produce inhibition, the speed and extent of inhibition being dependent on the multiplicity of infection.

(3) A virion protein is necessary to produce shut-off [9–11]. This fact is reinforced by the identification of poliovirus ts-mutants unable to cause shut-off. These map in the 5′-region of the genome known to code for coat proteins [12].

(4) The inhibition on host protein synthesis occurs at the level of initiation because host-cell polysomes break down after infection [13,14].

(5) Although some earlier results indicated that cellular mRNAs were inactivated following infection [14,15], it now seems clear that they remain stable [16,17].

(6) Cell-free systems from uninfected or virus-infected cells are both equally able to translate cellular or viral mRNAs in vitro [15,18], indicating that the observed in vivo specificity in the inhibition of the translation of host mRNAs is lost after preparation of the cell-free systems.

(7) In addition to host protein synthesis other cell functions also change: RNA synthesis is inhibited, the synthesis of lipids is enhanced and there are morphological changes in the infected cell, resulting in cell-death [1–3, 19]. All these alterations are known as the cytopathic effect [3,2]; it is not known if all have a common origin.

(8) Although these studies have been done mainly with picornavirus, similar results have also been obtained with a wide variety of animal viruses: Togaviruses, Rhabdoviruses, Adenoviruses, Herpesviruses and Poxviruses [2,3,19–21]. Shut-off seems to be a very widespread phenomenon in nature because it is also observed with bacteriophages [22]. As a general rule, maximal inhibition of host-cell protein synthesis occurs with viruses that eventually kill their host-cell during the later phases of infection, when viral coat protein is being made in large amounts. The shut-off phenomenon does not occur in Retroviruses, but these viruses are excluded from our model because their development does not kill the host-cell.

*On leave of absence from Instituto de Bioquímica de Macromoléculas, CSIC, Velazquez 144, Madrid 6, Spain

2. Explanations of the shut-off phenomenon

Several theories have been proposed during the years to explain the shut-off phenomenon, but to date none of them has been firmly established as correct. I will discuss some of these theories in the light of the observations described above.

The discovery that double-stranded RNA isolated from poliovirus-infected cells was an inhibitor of protein synthesis in vitro led to the suggestion that the replicative intermediate could be responsible for the observed specific inhibition of protein synthesis in vivo [23]. This theory has been discarded because it is now known that even in the absence of virus replication (i.e., without the production of ds RNA) shut-off still occurs [3]; poliovirus mutants in the replicase cause shut-off at the restrictive temperature [12]. Furthermore, the inhibition by ds RNA does not discriminate between the in vitro translation of viral and cellular mRNAs [24]. In addition, cell-free systems from infected cells are as active as controls in synthesizing cellular or viral proteins. This finding suggests that a stable inhibitor like ds RNA or a viral protein is not present in inhibitory amounts in the infected cell and is not responsible for shut-off.

It has been reported that the addition of large amounts of viral proteins inhibit translation in vitro [25]. The fact that such high concentrations of viral proteins are unlikely to be found in vivo, makes the possibility of a viral protein as a direct and specific inhibitor of the initiation of host mRNA translation highly unlikely. No specificity in the inhibition was described.

More recently it has been reported that viral mRNA could compete very efficiently with globin mRNA for in vitro translation and this could play a role in the explanation of the shut-off [15,26]. I have also observed that some viral mRNAs like polyoma 16 S RNA (which codes for VP1) also have a high rate of initiation as compared with actin mRNA (unpublished observations). The most plausible explanation for these results is that some viral mRNAs have a high affinity to bind to native 40 S ribosomal subunits and will result in a preferential translation of viral mRNAs when placed in competition with cellular mRNAs. I have also observed that the relative affinity to ribosomes between the viral and cellular mRNA is highly dependent on the concentration of potassium-ions in the cell-free system. But competition cannot be the complete explanation of shut-off, because if cellular mRNAs are simply replaced by more efficient viral mRNAs, why is total protein synthesis drastically inhibited? Why, even in the absence of viral mRNA production, does shut-off still occur? Why do the ts-mutants, that are unable to cause shut-off, map in the region of the genome which codes for coat protein? The inhibition of total protein synthesis after infection has to be produced by the generation of a real inhibitor, able to discriminate between host and viral translation.

The observation that a hypertonic medium inhibited specifically the initiation of cellular protein synthesis [27] but not the translation of viral mRNAs [28] has led to another model for shut-off which is in accordance with the classical observations outlined above. The model proposes that after infection an undefined nonspecific inhibitor of initiation is generated, and that the inhibitor has a differential effect on viral and cellular mRNAs, provided they have different initiation rates for translation [29].

3. The membrane-leakage model

Based on the classical observations and recent experiments that we have described [30], we proposed a different model which not only explains the shut-off phenomenon, but which may also explain the spectrum of different effects observed after viral infection. The model is summarized in the following steps (see fig.1):

(1) The virus attaches to the membrane, leaving a virus coat protein associated with the cellular membrane. The membrane-bound coat protein distorts the gradient of monovalent-ions which the membrane maintains.

(2) After decapsidation the free viral RNA directs the synthesis of progeny coat proteins initially in only small amounts. These are also inserted into the membrane. The viral replicase synthesized will produce more viral RNA.

(3) The translation of this progeny viral RNA will give rise to considerable amounts of coat proteins, some of which will be inserted in the membrane. These proteins continue to distort the gradient of monovalent-ions: sodium will leak in whereas potassium will leak out the cytoplasm. The result is an increase in the concentration of monovalent-ions inside the cell.

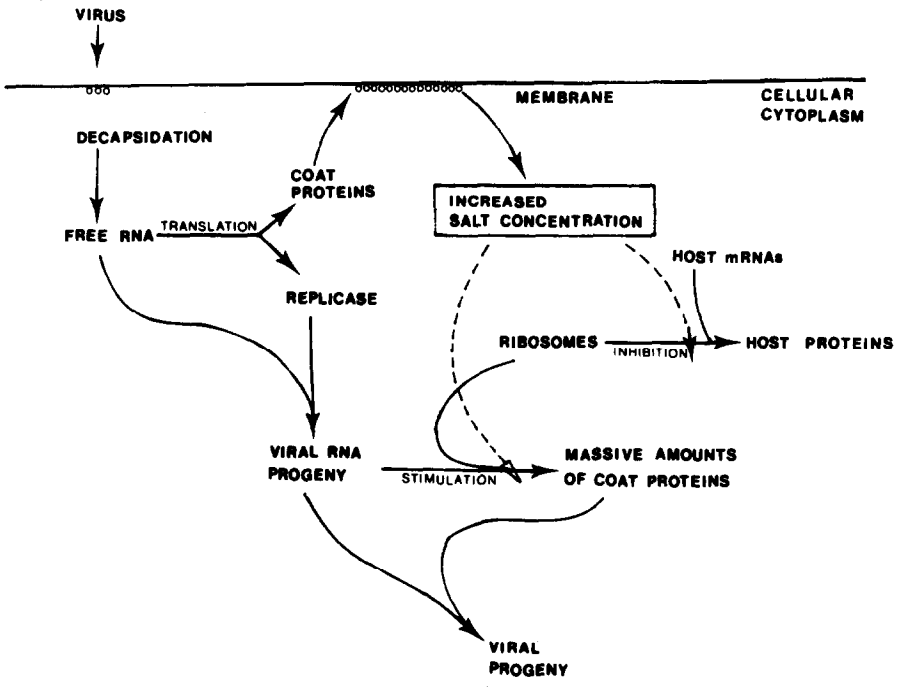

Fig.1. Schematic representation of the membrane-leakage model. The insertion into the membrane of a viral coat protein triggers its own synthesis by changing the concentration of ions in the cytoplasm. This effect is probably achieved by forming pores in the plasma membrane. The new ionic conditions created in the cytoplasm interfere with many cellular functions: i.e., protein synthesis is inhibited, whereas viral protein synthesis is stimulated. At the end of viral infection, the concentration of monovalent-ions will also be inhibitory for the initiation of viral protein synthesis, leaving the naked viral RNA ready for assembly with the coat proteins. The assembly process will take place under ionic conditions quite different from those existing in a normal cell.

(4) This imbalance in the concentration of ions could produce a variety of effects. In particular it will inhibit the initiation of host protein synthesis, whereas the initiation of translation of viral mRNAs will be stimulated. Other cellular functions might also be affected by the redistribution of other ions like magnesium or calcium [31].

The model is supported by in vitro experiments in which the addition of sodium to a cell-free system inhibited the initiation of translation of host protein synthesis, whereas it was stimulatory for the translation of picornavirus RNA [30] and other viral mRNAs, like Semliki Forest virus 26S RNA, polyoma 16S RNA and vesicular stomatitis virus mRNAs (Carrasco, Harvey and Smith, unpublished results). Furthermore we have indirect evidence that the cellular membrane becomes leaky to monovalent-ions when viral protein synthesis begins [30].

The model is consistent with all of the early observations on shut-off mentioned above. Thus virus particles are able to shut off even in the absence of replication [2,3]. Our model predicts that this occurs because a virion protein directly affects the membrane, causing the inhibition of host protein synthesis. It also predicts that the extent and the speed of the inhibition will be dependent on the multiplicity of infection, as observed experimentally [1–4]. The model is also able to explain the data obtained with ts-mutants, since if the mutated coat protein is unable to interact with the membrane and distort the gradient of monovalent-ions, then no inhibition of translation will occur.

In this model the influence of a viral coat protein on protein synthesis is indirect and hence relatively small amounts of such a protein are able to inhibit host protein synthesis totally. In addition, the mechanism I propose indicates that the coat protein stimulates its own synthesis via a membrane interaction: small

amounts of such a protein synthesized at the beginning of the viral-cycle interact with the membrane and make the cytoplasmic conditions much more suitable to synthesize viral proteins, which will in turn go to the membrane and so on (fig.1).

A key point in the explanation of the shutt-off phenomenon is the existence of a specific inhibitor of host mRNAs translation. I propose that such inhibitors are simply ions. The strongest support for such a proposition comes from our in vitro experiments in which we have described a specific discriminatory effect on translation by the addition of sodium: cellular mRNA translation was inhibited whereas viral translation was stimulated [30].

I wish to emphasize the difference between this model and the one proposed by Nuss and Koch [29]. As mentioned above these authors proposed that an indiscriminate inhibitor of initiation of viral and cellular mRNAs was generated after viral infection. If so, the virus-induced inhibitor will create conditions under which the translation of viral RNA will be suboptimal. In my model virus replication is more efficient in that the virus generates conditions inhibitory for the initiation of host protein synthesis. This leaves the protein-synthesizing apparatus available for viral protein synthesis and moreover, under these conditions, the viral mRNAs are translated more efficiently, because initiation on them is favoured. Analysing these ideas in an evolutionary context, one can imagine that viral evolution may have proceeded as follows: initially viral mRNAs simply competed out host mRNAs; hence, the viral mRNAs with higher efficiencies to bind to ribosomes were selected simply because viruses need to synthesize a given amount of coat protein for assembly, and viruses which make this in a shorter time will be favoured. This process has a limitation because the affinity of an mRNA to bind to a ribosome cannot increase indefinitely. If we now suppose that some viruses were able to create ionic conditions under which host mRNAs were unable to bind to ribosomes, the viral mRNAs more efficiently bound under the new conditions would be selected. The acquisition of such a property would make the virus more efficient and shorten the time needed for virus development. On the other hand the possibility of a cellular-response involving protein synthesis which interfered with virus-development would be diminished.

The model raises many questions: how can a viral protein modify the gradient of monovalent-ions? Are other viral mRNAs more efficiently translated under high ionic conditions? What other cellular and viral functions are affected by ionic changes? There are at least three ways in which a viral protein could modify the gradient of monovalent-ions:

(i) By inhibiting the enzyme involved in the establishment and conservation of this gradient: the Na^+/K^+ ATPase activity located on the membrane [32].

(ii) By acting as an ion-carrier ionophore [33].

(iii) By forming small pores in the lipid bilayer through which ions could diffuse freely as do the pore-forming ionophores [33].

To date I do not know of any published data which favours any one of these possibilities, but some of my unpublished experiments favour the third possibility.

One of the most important points in this model is that picornavirus mRNAs are more efficiently translated in vitro at high concentration of monovalent-ions; conditions under which the translation of cellular mRNA is restricted [30]. The structural molecular basis for this behaviour is still obscure. However a very striking recent result may offer a possible explanation; some viral mRNAs seem to have two or even three initiation codons in phase [34,35]. It has also been shown that picornavirus RNA has two potentially active initiation sites and the switch from one to another is achieved by changing the concentration of ions in vitro [36]. One possible explanation for the wider optima for monovalent-ions that the translation of some viral mRNAs display is that different initiation codons function under different salt conditions. They could generate the same mature protein if amino-terminal cleavage occurs after the more internal methionine coded for one of the potential initiation codons.

Acknowledgements

I wish to acknowledge Drs A. E. Smith and J. A. Melero for helpful discussions and help with the manuscript, and the Fundación Juan March for financial support.

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