Volume 3 · Number 2 · 1993
GENE EXPRESSION

MINI REVIEW

How does influenza virus regulate gene expression at the level of mRNA translation? Let us count the ways

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Influenza virus causes a disease that is responsible for up to 70,000 excess deaths a year in the United States and in epidemic years has caused up to 20,000,000 deaths worldwide (Murphy and Webster, 1990). It is thus of immediate importance to be able to attack the virus at many points in its life cycle. In addition to vaccines currently in use and vaccines in development that can be directed at non-surface proteins (Ulmer et al., 1993), the potential for drugs directed at influenza virus-specific mechanisms regulating gene expression could be significant. Additionally, influenza virus has proven to be an excellent model system for dissecting many mechanisms controlling eukaryotic gene expression. The influenza viruses types A and B are enveloped viruses with negative-stranded RNA genomes consisting of eight segments. In cells infected by influenza virus, host cell protein synthesis is severely diminished (Katze and Krug 1990, Krug et al., 1989). At the same time, viral mRNAs are efficiently and selectively translated (Katze and Krug, 1990). In the past several years, the molecular mechanisms underlying this translational control have begun to be elucidated. In this review, we will discuss the characterization of these mechanisms and the implications for the influenza virus life cycle as well as for normal cellular gene expression.

The interferon response: viral checks on cellular defenses

In order to completely appreciate the complexity of the approaches that influenza virus has evolved to ensure its continued protein synthesis during infection, it is useful to understand at least a small part of the interferon response with which many viruses must deal. Several eukaryotic viruses have developed strategies to avoid the decrease in protein synthetic rates that accompanies an unchecked interferon response. One of the cellular proteins synthesized during this response is the interferon-induced, dsRNA-activated protein kinase, now called PKR (for protein kinase RNA-activated), which we previously called P68 based on its M_r of 68,000 (Katze et al., 1991; Katze, 1992 and 1993; Hovanessian, 1991). This kinase has two distinct activities. The first activity is an autophosphorylation, or activation, reaction; the second is a protein kinase activity on its natural substrate, the α subunit of eukaryotic initiation factor 2 (eIF-2; Galabru and Hovanessian, 1987; Hovanessian, 1989). Phosphorylation of eIF-2α blocks the eIF-2B-mediated exchange of GDP in the inactive eIF-2-GDP complex. Since GTP is required for catalytic utilization of eIF-2 (Konieczny and Safer, 1983; Panniers and Henshaw, 1983; Safer,

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1983), this reaction leads to a limitation of functional eIF-2. Functional eIF-2 in the cell is normally required to bind initiator met-tRNA, via the ternary complex eIF-2-GTP-met-tRNA, to the initiating ribosomal subunit before mRNA is bound (Thach, 1991; Merrick, 1992). This limitation thus leads to an overall shutdown of protein synthesis in the infected cell, a situation not favorable for viruses, which must use the host cell translational machinery to synthesize proteins. Thus, many eukaryotic viruses have evolved mechanisms to downregulate the activity of PKR, and these have been thoroughly reviewed (Katze, 1992 and 1993; Katze and Krug, 1990).

The best characterized strategy to downregulate PKR is that evoked by adenovirus. In adenovirus infected cells, PKR is bound by the viral RNA polymerase III product VAI RNA, preventing activation of PKR and irreversibly inhibiting its enzymatic activities (Katze et al., 1987; Galabru et al., 1989; Ghadge et al., 1991; reviewed by Mathews and Shenk, 1991). Poliovirus has adopted an interesting strategy for inhibiting the activity of the kinase during infection. It has been shown that poliovirus does not prevent the activation of PKR (Black et al., 1989). Rather, poliovirus encodes a mechanism to cause the degradation of PKR via a proteolytic mechanism that is sensitive to both proteases and RNases and thus has an unusual multicomponent nature (Black et al., 1993).

A lean, mean protein machine: influenza virus encodes mechanisms to maintain the translational competence of host cells during infection so as to maximize viral protein synthesis

Influenza virus, like adenovirus and poliovirus, is insensitive to the translational inhibitory antiviral effects of interferon. The first experiments that indicated that influenza virus encoded one or more mechanisms to avoid the PKR-mediated antiviral effects of interferon used cells doubly infected with influenza virus and the adenovirus mutant *dl*331. *dl*331 does not encode the adenovirus VAI RNA and thus cannot prevent the activity of PKR in cells infected by *dl*331 alone. However, when *dl*331-infected cells were superinfected with influenza virus, a suppression of the protein kinase activity normally detected during *dl*331 infection was observed (Katze et al., 1986a). Further, the suppression of kinase activity was found in cells infected with influenza virus alone (Katze et al., 1988). These results have recently been independently confirmed (Feigenblum and Schneider, 1993).

To purify the PKR inhibitor biochemically, an in vitro assay, which quantitatively measured kinase inhibitory activity, was developed (Katze et al., 1988; Lee et al., 1990). Fractions from influenza virus-infected Madin-Darby bovine kidney cells were mixed with an interferon-treated 293 cell extract, which served as a source of PKR. The kinase was then immunoprecipitated and its activity measured by its ability to phosphorylate added histones (which mimics the phosphorylation of eIF-2α) in vitro in the presence of different fractions from the infected cell extracts. When done in the presence of radioactively labeled ATP, phosphorylation of the histones could be quantitated by scintillation counting.

The inhibitor was purified to near homogeneity using a protocol involving fractionation of the infected cell extracts by differential centrifugation, ammonium sulfate precipitation, passage over heparin agarose, Pharmacia FPLC Mono Q and Mono S column chromatography, and sedimentation through a glycerol gradient. The final product was visualized by silver staining as a single band of M_r 58,000 (Lee et al., 1990). It was found to inhibit both autophosphorylation of PKR and PKR phosphorylation of eIF-2α (Lee et al., 1992), which contrasts with the mechanisms of VAI inhibition of PKR in adenovirus infected cells, as VAI can only prevent autophosphorylation of PKR and must be present prior to the addition of activators of PKR to work effectively (Galabru et al., 1989). The inhibitor is not a protease, nor does it function as an ATPase, a ribonuclease, or a phosphatase in vitro (Lee et al., 1990).

Initially, it was hypothesized that the influenza virus inhibitor of PKR would be a viral gene product, based on analogy to VAI RNA activity in adenovirus-infected cells (Katze et al., 1986a and 1988). However, it was found that the inhibitor was not a viral gene product but was cellular in origin (Lee et al., 1990). This determination was based initially on a lack of reactivity with any influenza virus-specific antisera after partial purification (Lee et al., 1990), and finally and more directly on the purification of an identical inhibitor from uninfected cells. Western blot analysis using a peptide antibody revealed that the inhibitor was present

at equal levels in both influenza virus-infected and in mock-infected cells. Initially, in the uninfected cell extracts, inhibitory activity could not be detected in crude extracts or following a 100,000 × g centrifugation step. However, following ammonium sulfate precipitation of the extracts, inhibitory activity could be recovered (Lee et al., 1990). It is now hypothesized that the 58 kDa inhibitor of PKR is associated with its own inhibitory protein, called an anti-inhibitor, which becomes dissociated following either influenza virus infection or ammonium sulfate treatment of uninfected extracts, thus "unmasking" the inhibitory activity (Lee et al., 1992). Thus, the regulation of PKR and the mechanisms to keep overall translational levels high in the virus-infected cells will likely prove fertile ground for workers for much time to come.

Possible roles for the PKR and its inhibitor(s) in vivo

Until recently, both the anti-viral and anti-proliferative effects of interferon could be described only indirectly. Now, direct evidence for both of these effects suggests they may be causally related to the expression of PKR. For example, expression of PKR in mouse cells confers partial resistance to encephalomyocarditis virus growth (Meurs et al., 1992). Further, it appears that PKR plays a pivotal role in the normal regulation of gene expression (Barber et al., 1993a). PKR has been shown to be growth-supressive when expressed in yeast (Chong et al., 1992; Dever et al., 1993) and toxic when expressed in murine cells (Meurs et al., 1992) and even in insect cells (Barber et al., 1992). PKR may also be involved in adipocyte differentiation (Petryshyn et al., 1988) and perhaps in signal transduction (Mundschau and Faller, 1992).

Recent reports indicate that PKR may be a tumor-suppressor gene (Koromilas et al., 1992; Meurs et al., 1993). Evidence for this was obtained by expressing a functionally defective (inactivatable) kinase in mouse cells, which then displayed the phenotype of transformed cells. When these cells were injected into nude mice, the mice developed tumors, some in as few as five days (Meurs et al., 1992). Interestingly, the gene encoding the human PKR has been localized to chromosomal location 2p21-22 (Barber et al., 1993b), where clusters of nonrandom breakpoints and translocations have been found; these have been associated with—though not proven to be the cause of—various lipomas, lymphomas, and leukemias. It will be interesting to see if any of these specifically involve a defect in PKR, perhaps in its interactions with P58. Further work on the regulation of the expression and activities of both P58 and its own inhibitor will provide important information about the regulation of PKR not only in influenza virus-infected cells, but also in uninfected cells. These insights will be particularly important now, as it is probable that PKR is important in the normal regulation of gene expression. The necessity for tight regulation of PKR activity even in uninfected cells is further emphasized by subsequent reports of other cellular inhibitors of the kinase in human FL cells (Saito and Kawakita, 1991) and in mouse 3T3-F442A cells (Judware and Petryshyn, 1992), as well as in *ras* transformed cells (Mundschau and Faller, 1992). It will be of great interest to compare the structures and functions of these various inhibitors, and to define the mechanisms that are in common and those that are distinct, both in uninfected and in virus-infected cells. Thus, even nonvirologists will find significance in the studies of PKR and its inhibitors.

How does the host cell protein synthesis machinery discriminate between viral and cellular mRNAs?

In addition to ensuring that the overall translational capability of the cell is maintained throughout infection, influenza virus mediates the selective translation of influenza viral mRNAs (Katze et al., 1989; Katze and Krug, 1990). The mechanism of selective translation of viral mRNAs occurs in several viral systems, which we briefly review here before discussing influenza virus. The best understood strategies are those used by poliovirus and adenovirus. In both viral systems, the selective translation of viral mRNAs is assured by invoking cap-independent mechanisms for translational initiation. The inhibition of cellular protein synthesis in poliovirus-infected cells correlates with the degradation of P220, a component of the cap-binding protein complex called eukaryotic initiation factor 4F (eIF-4F; Etchison et al., 1982). In the absence of functional P220, cellular mRNAs, which initiate in a cap-dependent manner, cannot be translated, while the poliovirus mRNAs, which initiate cap-independently and internally (Pelletier and Sonenberg, 1988), con-

tinue to be translated. This is also the case for encephalomyocarditis virus (EMCV) mRNA translation (Jang and Wimmer, 1990; Witherell et al., 1993).

Translational initiation takes place in a stretch of mRNA called the ribosomal landing pad (RLP) in poliovirus or the internal ribosome entry site in EMCV. The precise events leading to the use of the RLP in poliovirus messages by the host cell's translational apparatus are just beginning to be understood and are now known to involve the La autoantigen (Meerovitch et al., 1993). The mechanism of selective translation of adenovirus mRNAs is less well understood but appears to be dependent on the dephosphorylation of eIF-4E (Huang and Schneider, 1991), which is also a component of the cap-binding complex eIF-4F. This functional limitation in eIF-4F allows only adenoviral mRNAs to be translated, as they are translated in a cap-independent manner and thus have little or no need for eIF-4E. It appears that specific elements in the 5′ untranslated region of adenoviral mRNAs, perhaps a relaxed secondary structure, allow these mRNAs to be translated during infection (Dolph et al., 1988; Zhang et al., 1989). For further review see Kozak (1986 and 1991).

What's so special about influenza viral mRNA selective translation?

What then is known about the regulation of viral and cellular protein synthesis in influenza virus-infected cells? Following infection, there is some down-regulation of cellular mRNA transcription – about a twofold decrease (Katze et al., 1984). There have been some reports (Inglis, 1982; Beloso et al., 1992) that influenza virus infection may lead to the destabilization and degradation of host cell mRNAs in the cytoplasm, particularly very late after infection. Most of the recent work, however, indicates that complex and clever translational controls are invoked in cells following influenza virus infection. It has been found that newly synthesized host cell mRNAs never reach the cytoplasm during infection (Katze and Krug, 1984). This is not due to a global shutoff of transport, but to a degradation of the host cell mRNAs in the nucleus. This degradation is probably the result of the cleavage of the 5′ ends (cap plus 10–13 nucleotides) of cellular RNA polymerase II transcripts (sometimes called cap-snatching or -stealing); these ends are then used to prime influenza viral mRNA synthesis (Krug, 1981). The decapped cellular mRNAs would then be more susceptible to degradation by cellular nucleases (Banerjee, 1980). However, this degradation of cellular mRNAs is not sufficient to explain the shutoff, because pre-existing cytoplasmic cellular mRNAs are stable and functional when tested in cell-free translation systems (Katze et al., 1986b). The block to translation occurs well before the maximal accumulation of viral messenger RNAs (Katze et al., 1986b), even during infection by temperature-sensitive influenza virus mutants, which accumulate only 10–20% of the RNA of the wild-type virus (G. Shapiro and R. Krug, unpublished data). Thus, the selective translation of the influenza viral mRNAs is not due to simple abundance of these mRNAs – a mechanism responsible for the preferential translation of viral mRNAs in other systems (for example, Walden et al., 1981). Rather, bona fide initiation and elongation blocks are exerted over cellular mRNAs, and influenza viral mRNAs escape these blocks. Finally, the nontranslated cellular mRNAs remain cytoskeleton-associated, as would ordinarily be expected only for translating mRNAs. This contrasts with the situation in poliovirus-infected cells, where cellular mRNAs become dissociated from the cytoskeleton (Lenk and Penman, 1979; Katze et al., 1989). Thus, unlike polioviral mRNAs, influenza virus mRNAs in the cytoplasm do not superficially appear distinguishable from cellular mRNAs to the host cell translational apparatus.

Early on during investigations of PKR, several experiments utilized cells co-infected with adenovirus and influenza virus. In the course of these experiments, it was noted that in cells doubly infected with adenovirus (which, as described above, exerts translational control over cellular protein synthesis) and influenza virus, influenza viral mRNAs not only overcome a general transport block imposed by adenovirus (Babich et al., 1983, Beltz and Flint 1979), but are translated as efficiently as in cells infected by influenza virus alone (Katze et al., 1986a, 1986b). Further, influenza virus is able to overcome a general translational defect in cells infected by *dl*331, an adenovirus mutant that cannot prevent the phosphorylation of PKR. In those cells, neither host nor viral protein synthesis occurs. Yet in cells doubly infected

by *dl*331 and influenza virus, influenza viral mRNAs continue to be translated (Katze et al., 1986a).

As noted above, the translational defect in *dl*331-infected cells stems from the inability of the virus to down-regulate the action of PKR, leading to phosphorylation of the α subunit of eIF-2, and thus to limitation of functional eIF-2. The limitation in functional eIF-2 causes the more competitive mRNAs to be translated at the expense of poorer ones, in this case the adenoviral and cellular mRNAs. Thus, despite the decided translational advantage enjoyed by adenovirus mRNAs over cellular mRNAs in cells infected by adenovirus alone, influenza viral mRNAs are still translated in the doubly infected cells. Furthermore, that influenza virus-specific mRNAs are translated at all in the *dl*331-infected cells indicates that influenza virus exerts strong translational controls of its own during infection.

Which particular controls were being used was at one point a complete mystery. However, the results of the double infection experiments, as well as the results in influenza virus-infected cells, provided indirect evidence that the *structure* of the influenza viral mRNAs contains features that allow them to initiate selectively and efficiently in infected cells. To begin to look at the importance of mRNA structure directly, the influenza nucleocapsid protein (NP) mRNA was expressed in the absence of any other influenza viral gene products by inserting the cDNA into an adenoviral vector under the control of the major late promoter (Alonso-Caplen et al., 1988). Following infection by the recombinant adenovirus, the NP mRNA was found on polysomes similar in size to those in cells infected by influenza virus alone, or in cells doubly infected by influenza virus and adenovirus. Thus, the selective translation of influenza viral mRNAs is independent of any influenza gene products, except perhaps NP itself.

To demonstrate directly that mRNA structure is at least partly responsible for the selective translation of the influenza viral mRNAs, a transfection/infection assay was developed in which representative viral or cellular cDNAs were transfected into COS-1 cells, which were then infected with influenza virus (Garfinkel and Katze, 1992). With cellular genes, such as secreted embryonic alkaline phosphatase (SEAP) or interleukin-2 (IL-2), which are not expressed endogenously in COS-1 cells, it was found that SEAP protein expression, as measured by either alkaline phosphatase activity from culture medium or by radiolabeling and immunoprecipitation, is inhibited following influenza virus infection. IL-2 protein expression, measured by radioimmunoprecipitation, is also subject to host cell shutoff following influenza virus infection. In marked contrast, a shortened version of the influenza viral NP gene (NP-S, containing an in-frame deletion of 255 nucleotides), which is distinguishable from the viral NP on SDS/polyacrylamide gels, is not subject to host cell shutoff, and synthesis of NP-S occurs equally efficiently in both influenza virus-infected and in uninfected cells. Northern blot analysis of exogenous cellular mRNAs demonstrated that the shutoff of SEAP and IL-2 gene expression is at the level of translation, while polysome analysis indicated that the blocks to protein synthesis occur at both the initiation and elongation stages of protein synthesis. Thus, this assay faithfully reproduced the result of the influenza virus-mediated host cell shutoff of protein synthesis. More importantly, the results provided direct evidence that the structure of the viral mRNAs is important in mediating their selective translation, as these mRNAs were expressed independently of any influenza viral replication machinery. Further, the only differences between the exogenous cellular mRNAs and the influenza viral mRNAs were the actual untranslated and coding sequences; sequences derived from the transfection vector were identical for every construct.

Which structural features of the viral mRNA allow it to avoid the host cell shutoff? Because of the many studies emphasizing the importance of the cap and the 5′ untranslated region (5′-UTR) of both cellular mRNAs (for examples, see Kozak, 1991) and, as indicated above, viral mRNAs (for example, Dolph et al., 1988; Zhang et al., 1989; Pelletier and Sonenberg, 1988; for review, Kozak, 1991), we wanted to determine first whether the selective translation of influenza viral mRNAs occurs via a cap-dependent mechanism, or in a cap-independent manner, as had been found for poliovirus and adenovirus. To define the initiation mechanism, cells were transfected with either IL-2 or NP-S cDNAs, then infected by poliovirus. In both cases, protein synthesis from these mRNAs was severely inhibited. Thus, viral NP-S mRNA,

which is completely refractory to the host cell shutoff in influenza virus-infected cells, is as sensitive to the poliovirus host cell shutoff as exogenous cellular mRNAs are. To confirm and extend these results, cells were infected with influenza virus and then superinfected by poliovirus. It was found that essentially all influenza viral protein synthesis was blocked; again, this occurred at the level of translation, as influenza viral mRNAs were stable throughout the assay. Essentially the same result was obtained by infecting cells first with poliovirus and then with influenza virus (Garfinkel and Katze, 1992) as by co-infecting cells with influenza virus and poliovirus simultaneously (Schrader and Westaway, 1990). In sum, unlike the cap-independent initiation of translation used by adenovirus and poliovirus to mediate the selective translation of mRNAs, influenza virus mRNAs, like most cellular mRNAs, are completely dependent upon the presence of a cap structure to allow translational initiation. This is one of very few reported cases of cap-dependent selective translation of viral mRNAs. Others include vesicular stomatitis virus (Berg and Grinnell, 1993) and vaccinia virus, which may induce selective translation of viral mRNAs by preventing cellular mRNAs from associating with poly(A)-binding protein (Bablanian et al., 1991).

Musings on mechanisms

We must now address the question of which specific mechanisms mediate the selective translation of influenza viral mRNAs in infected cells. Since initiation of influenza viral mRNA translation occurs in a cap-dependent manner, and presumably is not initiated internally, it is probable that a specific—but still unidentified—primary sequence or higher order structure mediates selective translation of the viral mRNAs in infected cells.

The 5′ untranslated region (UTR) of both viral and cellular mRNAs has often been found to contain critical sequences that regulate mRNA translation. Among the many examples of highly structured UTRs resulting in a block to ribosomal scanning—and thus down-regulation of translation initiation—are the c-*sis*/PDGF mRNA (Rao et al., 1988) and other proto-oncogene and growth factor mRNAs. Further, several proto-oncogenes, such as *lck* (Marth et al., 1988), have upstream AUGs which result in improper initiation and thus reduction of synthesis of the appropriate polypeptide products (for an extensive review, see Kozak, 1991 and 1993).

As noted above, adenoviral mRNAs may contain structural elements leading to increased translation during infection. It is important to note again that the 5′ ends of influenza viral mRNAs are derived from host cell polymerase II transcripts (Krug, 1981). Further, at least in vitro, the virus seems to prefer 5′ ends of capped mRNAs with reduced secondary structure (Bouloy et al., 1978). Since a shorter secondary structure is associated with enhanced translational efficiency (Kozak, 1991), it has been argued that influenza steals the caps of the best translated cellular mRNAs (Krug et al., 1980), although there is no proof for this mechanism working in vivo. Additionally, the NP cDNA used in the transfection/infection assay did not contain any host cell sequences but was nevertheless translated in virally infected cells. The influenza viral RNAs also contain a common 12 or 13 nucleotide sequence directly downstream from the stolen 5′ ends in vivo (Skehel and Hay, 1978), known to be important in viral transcription, replication, and virion assembly (Luytjes et al., 1989). Since it also occurs in every mRNA, this sequence may confer at least some translational selectivity on the influenza viral mRNAs. If this element were entirely responsible for mediating selective translation, all the viral mRNAs should be translated with equal efficiency throughout the infection; but this has not been shown to be the case (Skehel and Hay, 1978; Katze et al., 1986b; Yamanaka et al., 1991). Moreover, it is important to note that by the late stage of infection, the only proteins being synthesized are influenza viral proteins, albeit with different efficiencies. This sequence and the rest of the viral NP 5′ untranslated region—which together consist of only 45 nucleotides—and other influenza viral 5′ UTRs are currently being tested for their ability to impart translational regulation to heterologous mRNAs during influenza virus infection.

Defining the precise sequences in the influenza viral 5′ UTRs responsible for the translational control exerted during infection will be the first step in describing the complete mechanism of this control. Whether these elements function only through their primary or higher order structures, or, more likely, by interacting with some factor, either viral or cellular, remains to be determined. Numerous models can

be hypothesized to explain these interactions. For example, it was recently demonstrated (Feigenblum and Schneider, 1993) that eIF-4E, the cap-binding protein, is somewhat dephosphorylated during influenza virus infection. Dephosphorylation of eIF-4E leads to a functional limitation in eIF-4F, one activity of which is to unwind secondary structure in the 5′ UTRs of mRNAs (Thach, 1991). Because eIF-4E is already limiting in eukaryotic cells (Lazaris-Karatzas et al., 1990; Thach, 1991), it can be hypothesized that the 5′ UTR of influenza viral mRNAs are somehow more attractive to this initiation factor, especially late in infection. This possibility can initially be tested by standard RNA–protein interaction assays, such as gel shift and UV-crosslinking analyses. The interaction of the viral mRNA sequences with other factors, either viral or cellular, can be tested in a similar way.

Homage to influenza virus: how can eight RNA segments do so much?

In addition to the translational mechanisms that influenza virus invokes to ensure the efficient and selective production of virus-specific proteins, the virus has also evolved several other posttranscriptional processes that lead both to an increase in the coding capacity of the genome and to additional levels of potential regulation of gene expression. All of these mechanisms, including translational regulation, are summarized in Table 1. For example, influenza viruses have been found to use mechanisms such as bicistronic mRNAs (Shaw et al., 1983; Williams and Lamb, 1986 and 1989), overlapping reading frames (Lamb and Lai, 1980; Nakada et al., 1986), controlled splicing and nucleocytoplasmic transport (Plotch and Krug, 1986; Alonso-Caplen and Krug, 1991; Alonso-Caplen et al., 1992), and coupled translation of tandem cistrons (Horvath et al., 1990) to diversify the proteins produced from the NS1 and M1 mRNAs. Influenza virus has not been shown to use non-AUG initiation codons, as is the case for some viruses (Cattaneo, 1989), nor does it appear to employ frameshifting to increase its coding capacity, as do retroviruses and coronaviruses (Jacks et al., 1988; Brierley et al., 1989). For a more in-depth review of these posttranscriptional processes, the reader is directed to the fine and comprehensive overviews by Lamb (1990) and Lamb and Horvath (1991).

Influenza virus also appears to carry out some temporal regulation of gene expression at the level of translation. Yamanaka et al. (1988 and 1991) studied this putative temporal regulation

Table 1. Posttranscriptional mechanisms leading to the efficient and selective translation of influenza viral mRNAs in infected cells

Strategy	*References*
Inhibition of cellular mRNA transport/degradation of mRNAs in nucleus	Katze and Krug, 1984
Inhibition of the interferon-induced, double-stranded RNA-activated protein kinase, PKR	Katze et al., 1986a, 1988 Lee et al., 1990, 1992 Feigenblum and Schneider, 1993
Cap-dependent, selective translation of influenza viral mRNAs	Katze et al., 1986b Alonso-Caplen et al., 1988 Garfinkel and Katze, 1992 Garfinkel and Katze, unpublished data
Inhibition of cellular mRNA translation at initiation and elongation stages	Katze et al., 1986b Garfinkel and Katze, 1992
Structure of influenza viral mRNAs	Alonso-Caplen et al., 1988 Garfinkel and Katze, 1992
Dephosphorylation of eukaryotic initiation factor 4E	Feigenblum and Schneider, 1993
Temporal control of influenza viral protein synthesis	Yamanaka et al., 1988, 1991
Bicistronic mRNAs (influenza virus type B)	Shaw et al., 1983 Williams and Lamb, 1986, 1989
Spliced mRNAs/Overlapping reading frames	Lamb and Lai, 1980 Briedis and Lamb, 1982 Nakada et al., 1986
Controlled nucleocytoplasmic transport of spliced mRNAs	Plotch and Krug, 1986 Alonso-Caplen and Krug, 1991 Alonso-Caplen et al., 1992

by transfecting HeLa cells with a CAT reporter gene appended to the 5′ untranslated region of each of the viral mRNAs separately and then infected them with influenza virus. They looked for CAT activity at early and late times after infection and demonstrated that the 5′ UTRs of the mRNAs encoding polypeptides—such as the nonstructural protein, required early in infection, and the neuraminidase protein, required late in infection—were utilized at the same times that the viral proteins were detected, even though they were expressed independently of the influenza virus replication machinery. Understanding the mechanisms responsible for this sort of regulation will most likely also yield information on the regulation of selective mRNA translation, as discussed above.

Like many RNA viruses, influenza virus has evolved such that its genome size has apparently been minimized (Strauss et al., 1990). Influenza virus has developed several mechanisms to ensure that this size restriction does not interfere with its capability to produce large numbers of infectious virions; many of these mechanisms exist totally outside the realm of translational regulation that we have discussed here. For example, influenza virus is (in)famous for its ability to undergo antigenic drifts and shifts in order to avoid the host immune response (Smith and Palese, 1989).

In sum, influenza virus has evolved a variety of strategies which lead to high production of virus in infected cells. An important step is the regulation of translation both globally and selectively, leading to the efficient production of influenza virus-specific proteins at the expense of cellular protein synthesis. Continued studies on these forms of regulation will be greatly enhanced by the use of recombinant influenza viruses and the reverse genetics described by Palese and coworkers (Luytjes et al., 1989; Enami et al., 1990) and Seong and Brownlee (1992). Ultimately, understanding the mechanisms that allow influenza virus to convince the cell that its viral mRNAs are worthy of being translated while the host cellular mRNAs are ignored will almost certainly reveal profound insights into the normal regulation of cellular mRNA translation.

Acknowledgments

We acknowledge the members of the Katze laboratory who, in addition to the authors, have carried out much of the work described herein: Glen Barber, Tracy Black, Tae Gyu Lee, Judy Tomita, and Marlene Wambach.

The work presented from the authors' laboratory was supported by National Institutes of Health grants AI-22646 and RR-00166 to M. G. Katze, and by a Molecular and Cellular Biology Training Grant from the National Institute of General Medical Sciences, National Research Service Award T32-GM07270, to M. S. Garfinkel.

References

F. V. Alonso-Caplen and R. M. Krug (1991), Mol Cell Biol 11, 1092–1098.

F. V. Alonso-Caplen, M. G. Katze, and R. M. Krug (1988), J Virol 62, 1606–1616.

F. V. Alonso-Caplen, M. E. Nemeroff, Y. Qiu, and R. M. Krug (1992), Genes Dev 6, 255–267.

R. Bablanian, S. K. Goswami, M. Esteban, A. K. Banerjee, and W. C. Merrick (1991), J Virol 65, 4449–4460.

G. N. Barber, J. Tomita, M. S. Garfinkel, A. G. Hovanessian, E. Meurs, and M. G. Katze (1992), Virology 191, 670–679.

G. N. Barber, M. Wambach, M.-L. Wong, T. E. Dever, A. G. Hinnebusch, and M. G. Katze (1993a), Proc Natl Acad Sci USA 90, 4621–4625.

G. N. Barber, S. Edelhoff, M. G. Katze, and C. M. Disteche (1993b), Genomics 16, 765–767.

A. Beloso, C. Martínez, J. Valcárcel, J. F. Santarén, and J. Ortín (1992), J Gen Virol 73, 575–581.

D. T. Berg and B. W. Grinnell (1992), Biochem Biophys Res Comm 189, 1585–1590.

T. L. Black, B. Safer, A. Hovanessian, and M. G. Katze (1989), J Virol 63, 2244–2252.

T. L. Black, G. N. Barber, and M. G. Katze (1993), J Virol 67, 791–800.

D. J. Briedis and R. A. Lamb (1982), J Virol 42, 186–193.

I. P. Brierly, P. Digard, and S. C. Inglis (1989), Cell 57, 537–547.

R. Cattaneo (1989), Trends Biochem Sci 14, 165–167.

K. L. Chong, K. Schappert, E. Meurs, F. Feng, T. F. Donahue, J. D. Friesen, A. G. Hovanessian, and B. R. G. Williams (1992), EMBO J 11, 1553–1562.

T. E. Dever, J.-J. Chen, G. N. Barber, A. M. Cigan, L. Feng, T. F. Donahue, I. M. London, M. G. Katze, and A. G. Hinnebusch (1993), Proc Natl Acad Sci USA 90, 4616–4620.

P. J. Dolph, J. Juang, and R. J. Schneider (1990), J Virol 64, 2669–2677.

M. Enami, W. Luytjes, M. Krystal, and P. Palese (1990), Proc Natl Acad Sci 87, 3802–3805.

D. Etchison, S. Milburn, I. Edery, N. Sonenberg, and J. W. B. Hershey (1982), J Biol Chem 257, 14806–14810.

D. Feigenblum and R. J. Schneider (1993), J Virol 67, 3027–3035.
J. Galabru and A. G. Hovanessian (1987), J Biol Chem 262, 15538–15544.
M. S. Garfinkel and M. G. Katze (1992), J Biol Chem 267, 9383–9390.
G. D. Ghadge, S. Swaminathan, M. G. Katze, and B. Thimmapaya (1991), Proc Natl Acad Sci USA 88, 7140–7144.
J. Huang and R. J. Schneider (1991), Cell 65, 271–280.
S. C. Inglis (1982), Mol Cell Biol 2, 1644–1648.
T. Jacks, H. D. Madhani, F. R. Masiarz, and H. E. Varmus (1988), Cell 55, 447–458.
S. K. Jang and E. Wimmer (1990), Genes Dev 4, 1560–1572.
R. Judware and J. Petryshyn (1992), J Biol Chem 267, 21685–21690.
M. G. Katze (1992), J Interferon Res 12, 241–248.
M. G. Katze (1993), Semin Virol 4, 508.1–508.10.
M. G. Katze and R. M. Krug (1984), Mol Cell Biol 4, 2198–2206.
M. G. Katze and R. M. Krug (1990), Enzyme 44, 265–277.
M. G. Katze, T. Y. Chen, and R. M. Krug (1984), Cell 37, 483–490.
M. G. Katze, B. M. Detjen, B. Safer, and R. M. Krug (1986a), Mol Cell Biol 6, 1741–1750.
M. G. Katze, D. DeCorato, and R. M. Krug (1986b), J Virol 60, 1027–1039.
M. G. Katze, J. Tomita, T. Black, R. M. Krug, B. Safer, and A. G. Hovanessian (1988), J Virol 62, 3710–3717.
M. G. Katze, J. Lara, and M. Wambach (1989), Virology 169, 312–322.
M. G. Katze, M. Wambach, M.-L. Wong, M. Garfinkel, E. Meurs, K. Chong, B. R. G. Williams, A. G. Hovanessian, and G. N. Barber (1991), Mol Cell Biol 11, 5497–5505.
A. Konieczny and B. Safer (1983), J Biol Chem 256, 3402–3408.
A. E. Koromilas, S. Roy, G. N. Barber, M. G. Katze, and N. Sonenberg (1992), Science 257, 1685–1689.
M. Kozak (1986), Adv Virus Res 31, 229–292.
M. Kozak (1991), J Cell Biol 115, 887–903.
M. Kozak (1992), Annu Rev Cell Biol 8, 197–225.
R. M. Krug (1981), Curr Top Microbiol Immunol 93, 125–150.
R. M. Krug, B. A. Broni, A. J. Lafiandra, M. A. Morgan, and A. J. Shatkin (1980), Proc Natl Acad Sci USA 77, 5874–5878.
R. M. Krug, F. Alonso-Caplen, I. Julkunen, and M. G. Katze (1989), in The Influenza Viruses (R. M. Krug, ed.), Plenum Press, New York, pp. 89–152.
R. A. Lamb (1989), in The Influenza Viruses (R. M. Krug, ed.), Plenum Press, New York, pp. 1–87.
R. A. Lamb and C. M. Horvath (1991), Trends Genet 7, 261–266.
R. A. Lamb and C.-J. Lai (1980), Cell 21, 475–485.
A. Lazaris-Karatzas, K. S. Montine, and N. Sonenberg (1990), Nature 345, 544–547.
T. G. Lee, J. Tomita, A. G. Hovanessian, and M. G. Katze (1990), Proc Natl Acad Sci USA 87, 6208–6212.
T. G. Lee, J. Tomita, A. G. Hovanessian, and M. G. Katze (1992), J Biol Chem 267, 14238–14243.
R. Lenk and S. Penman (1979), Cell 16, 289–301.
W. Luytjes, M. Krystal, M. Enami, J. D. Pavin, and P. Palese (1989), Cell 59, 1107–1113.
J. D. Marth, R. W. Overell, K. E. Meier, E. G. Krebs, and R. M. Perlmutter (1988), Nature 332, 171–173.
M. B. Mathews and T. Shenk (1991), J Virol 65, 5657–5662.
K. Meerovitch, Y. V. Svitkin, H. S. Lee, F. Lejbkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg (1993), J Virol 67, 3798–3807.
W. C. Merrick (1992), Microbiol Rev 56, 291–315.
E. Meurs, Y. Watanabe, S. Kadereit, G. N. Barber, M. G. Katze, K. Chong, B. R. G. Williams, and A. G. Hovanessian (1992), J Virol 66, 5805–5814.
E. F. Meurs, J. Galabru, G. N. Barber, M. G. Katze, and A. G. Hovanessian (1993), Proc Natl Acad Sci USA 90, 232–236.
L. J. Mundschau and D. V. Faller (1992), J Biol Chem 267, 23092–23098.
B. R. Murphy and R. G. Webster (1990), in Virology (B. N. Fields and D. M. Knipe, eds.), Raven Press, New York, pp. 1091–1173.
S. Nakada, P. N. Graves, and P. Palese (1986), Virus Res 4, 263–273.
R. Panniers and E. C. Henshaw (1983), J Biol Chem 258, 7928–7934.
J. Pelletier and N. Sonenberg (1988), Nature 334, 320–325.
R. Petryshyn, J.-J. Chen, and I. M. London (1988), Proc Natl Acad Sci 85, 1427–1431.
S. J. Plotch and R. M. Krug (1986), Proc Natl Acad Sci USA 83, 5444–5448.
C. D. Rao, M. Pech, K. C. Robbins, and S. A. Aaronson (1988), Mol Cell Biol 8, 284–292.
B. Safer (1983), Cell 33, 7–8.
S. Saito and M. Kawakita (1991), Microbiol Immunol 35, 1105–1114.
A. P. Schrader and E. G. Westaway (1990), Arch Virol 114, 75–89.
B. L. Seong and G. G. Brownlee (1992), Virology 186, 247–260.
M. W. Shaw, P. W. Choppin, and R. A. Lamb (1990), Proc Natl Acad Sci USA 80, 4879–4883.
J. J. Skehel and A. J. Hay (1978), Nucleic Acids Res 5, 1207–1219.
F. I. Smith and P. Palese (1989), in The Influenza

Viruses (R. M. Krug, ed.), Plenum Press, New York, pp. 319–359.

E. G. Strauss, J. H. Strauss, and A. J. Levine (1990), in Virology (B. N. Fields and D. M. Knipe, eds.), Raven Press, New York, pp. 167–190.

J. B. Ulmer, J. J. Donnelly, S. E. Parker, G. H. Rhodes, P. L. Felgner, V. J. Dwarki, S. H. Gromkowski, R. R. Deck, C. M. DeWitt, A. Friedman, L. A. Hawe, K. R. Leander, D. Martinez, H. C. Perry, J. W. Shiver, D. L. Montgomery, and M. A. Liu (1993), Science 259, 1745–1749.

W. E. Walden, T. Godefrey-Colburn, and R. E. Thach (1981), J Biol Chem 256, 11739–11746.

M. A. Williams and R. A. Lamb (1986), Mol Cell Biol 6, 4317–4328.

M. A. Williams and R. A. Lamb (1989), J Virol 63, 28–35.

G. W. Witherell, A. Gil, and E. Wimmer (1993), Biochemistry, in press.

K. Yamanaka, A. Ishihama, and K. Nagata (1988), Virus Genes 2, 19–30.

K. Yamanaka, K. Nagata, and A. Ishihama (1991), Arch Virol 120, 33–42.

Y. Zhang, P. J. Dolph, and R. J. Schneider (1989), J Biol Chem 264, 10679–10684.