Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ADVANCES IN VIRUS RESEARCH, VOL. 31

REGULATION OF PROTEIN SYNTHESIS IN VIRUS-INFECTED ANIMAL CELLS

Marilyn Kozak

Department of Biological Sciences
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

I. Introduction

Historically, a number of "firsts" in the field of eukaryotic translation were scored with plant and animal viruses. These include the discovery of the unique initiator met-tRNA (Smith and Marcker, 1970), the realization that most eukaryotic mRNAs are functionally monocistronic (Jacobson and Baltimore, 1968; Shih and Kaesberg, 1973), sequencing of the first eukaryotic ribosome binding site (Dasgupta *et al.*, 1975), the existence and function of the 7-methylguanosine (m^7G) cap (Furuichi *et al.*, 1975), and the first evidence for "scanning" during initiation (Kozak and Shatkin, 1978a). The analysis of viral mRNAs has revealed not only the aforementioned general rules, but also ways in which the rules are occasionally skirted. This is important inasmuch as one cannot deduce the shape of a room by standing in the center and waving a small flashlight; one has to explore the corners and crevices. Studies carried out with viral mRNAs have taught us that eukaryotic ribosomes can (albeit rarely) initiate on uncapped mRNAs, use two initiation sites in the same mRNA, initiate at a codon other than AUG, read through a terminator codon, shift reading frames during elongation, and reinitiate after translating the 5′-proximal cistron. To carry out these feats with viral mRNAs, the cells' translational machinery has to operate on the fringes of the rules, but the machinery usually does not have to be altered. I will justify that view in the following pages. For now the simplest evidence to invoke is that, although the above "anomalies" were first detected in virus-infected cells, most of them have been reproduced with appropriately engineered genes introduced into uninfected cells or cell-free extracts. Because viruses stretch but probably do not rewrite the rules, we have learned much about the normal workings of the translational machinery from the study of viral protein synthesis.

What follows is first a summary of structural features that govern

the translation of viral mRNAs: *where* the synthesis of a protein starts and ends, *how many* proteins can be produced from one mRNA, and *how efficiently*. The next section focuses on the interplay between viral and cellular mRNAs and the translational machinery. That interplay, together with the intrinsic structure of viral mRNAs, determines the patterns of translation in infected cells. The final section points out some possibilities for translational regulation that can only be glimpsed at present, but are likely to come into focus in the future.

To keep the project manageable, I have concentrated on animal viruses. Plant viruses are mentioned, however, when they provide the best (or sometimes the unique) example of a given mechanism.

II. Intrinsic Features That Regulate the Translation of Viral mRNAs

A. Overview

The structural requirements for mRNA function have been determined by inspection of natural eukaryotic mRNAs, followed by manipulation of features that looked suspicious. The general structural characteristics of eukaryotic mRNAs have been reviewed previously (Kozak, 1983a) and will not be elaborated here. The discovery of the m^7G cap on a wide variety of viral and cellular mRNAs (Shatkin, 1976) was a provocative clue that the mechanism of initiation in eukaryotes differs from prokaryotes. Although the list of plant virus mRNAs that are translated without a cap has grown in recent years, picornaviruses and caliciviruses are still the only animal viruses known to be translated without a cap (Nomoto *et al.*, 1976; Ehresmann and Schaffer, 1979). Indeed, the near-indispensibility of the m^7G cap may be inferred from the fact that animal viruses that replicate in the cytoplasm routinely encode their own capping and methylating enzymes. This is true not only for poxviruses (Moss *et al.*, 1976), where the vast coding capacity of the genome allows room for frills, but also for reovirus (Furuichi *et al.*, 1976), vesicular stomatitis virus (VSV) (Abraham *et al.*, 1975), and alphaviruses (Cross, 1983), in which the small size of the genome limits the encoded proteins to the barest essentials. The m^7G cap enhances both the stability and translatability of mRNAs. Transcripts that are capped but not methylated are stable, but nonetheless untranslatable (Furuichi *et al.*, 1977; Horikami *et al.*, 1984).

Much of the discussion that follows assumes that a scanning mechanism underlies the initiation process. The scanning model postulates that a 40 S ribosomal subunit binds initially at the 5′ end of the mRNA

and migrates until it reaches the first AUG triplet. If the first AUG codon occurs in the optimal context (ACCAUGG—see Kozak, 1981a, 1984a, 1986a) all 40 S subunits stop there, and that AUG serves as the unique site of initiation. If the first AUG triplet occurs in a suboptimal context, only some 40 S subunits will initiate there; some will migrate beyond that site and initiate at an AUG codon farther downstream. The scanning hypothesis is not universally accepted, but it is supported by extensive evidence from many laboratories (reviewed by Kozak, 1980, 1981b, 1986a). Two alternative models have been suggested from time to time. One is that ribosomes bind directly to the sequence around the AUG codon, but experiments designed to distinguish between scanning and direct binding do not support the latter (Kozak, 1979a, 1983b). A hybrid mechanism in which 98% of the ribosomes scan from the 5′ end, while 2% of the binding occurs directly at the AUG start site, is difficult to rule out, however. Another suggestion is that secondary structure might guide the choice of AUG codons (this idea is evaluated a few paragraphs hence).

One consequence of the scanning mechanism is that deleting the "ribosome binding site" (i.e., the normal initiator codon and flanking sequences) will not abolish translation; ribosomes will simply use the next AUG codon downstream, which, in some cases, has been shown to direct the synthesis of a biologically active, truncated protein (Downey *et al.*, 1984; Halpern and Smiley, 1984; Katinka and Yaniv, 1982). Conversely, introducing spurious upstream AUG codons will reduce initiation from the authentic start site—a prediction that has been verified many times with laboratory constructs (Bandyopadhyay and Temin, 1984; Lomedico and McAndrew, 1982; Smith *et al.*, 1983; Zitomer *et al.*, 1984) as well as with naturally occurring variant forms of mRNA from the early[1] and late regions of simian virus 40 (SV40) (Barkan and Mertz, 1984). When the context around an upstream AUG codon conforms closely to the ACCAUGG consensus sequence, initiation from the downstream site is suppressed almost completely (Kozak, 1983b, 1984b; Liu *et al.*, 1984; M. Scott and H. Varmus, personal communication). When the context around the upstream AUG

[1] Transcription of the SV40 early region continues at late times, but there is a shift in the promoter; the "late–early" transcripts therefore have longer 5′-noncoding sequences (Buchman *et al.*, 1984; Ghosh and Lebowitz, 1981). George Khoury and his colleagues have found that the "early–early" form of SV40 mRNA, in which the leader sequence has no spurious AUG codons, can be translated *in vitro* about 10-fold more efficiently than a late–early transcript that has two upstream AUG codons. The 23-amino acid peptide that is encoded in a minicistron near the 5′ end of late–early mRNA has actually been detected *in vitro* and *in vivo* (G. Khoury, personal communication), thus validating the interpretation that synthesis of T antigen from late–early mRNA is inefficient because some 40 S ribosomal subunits are deflected by the upstream AUG codons.

codon is less ideal, initiation from the downstream site is reduced but not abolished (Kozak, 1986a). Stated in a more positive way, when the 5′-proximal AUG codon occurs in a suboptimal context, ribosomes are able to initiate at the first *and the second* AUG codons. This "leaky" scanning process is further explained and documented in Section II,C.

The scanning mechanism predicts that translation should be down-regulated by any ploy that interferes with the linear movement of 40 S ribosomal subunits from the cap to the AUG codon: binding of a protein to the 5′-noncoding sequence; introducing spurious out-of-frame AUG codons, as mentioned above; annealing cDNA fragments that are complementary to the 5′-untranslated sequence (Haarr *et al.*, 1985; Perdue *et al.*, 1982; Privalsky and Bishop, 1982; Willis *et al.*, 1984); or creating a stable hairpin anywhere upstream from the AUG codon, as described in the next section. On the other hand, the simplicity of the scanning mechanism suggests few possibilities for enhancing translation. Although we know what features should be absent from the leader for a message to be efficient, the only features known to contribute in a positive way are the m^7G cap and the sequence directly flanking the initiator codon. A promising place to look for other positive effectors is the tripartite leader on late adenovirus mRNAs. Transposition of the 200-nucleotide tripartite leader sequence to heterologous mRNAs stimulates their translation 20-fold (Berkner and Sharp, 1985; Logan and Shenk, 1984), but the feature responsible for the stimulation has not been pinpointed, and could turn out disappointingly to be a long sequence that simply lacks all of the negative effectors cited above. The impression that the leader sequences on most viral mRNAs do not contain unidentified translational "enhancers" is reinforced by the ease with which 5′-noncoding sequences can be deleted without deleterious effects (Bendig *et al.*, 1980; Spindler and Berk, 1984a; Villarreal *et al.*, 1979).[2] If our intuition is correct that "extra" 5′-noncoding sequences are more likely to inhibit than to help, the trend toward short 5′-noncoding sequences on many viral mRNAs becomes significant (reviewed by Kozak, 1981b; see also Rosel and Moss, 1985). Indeed, the 24-nucleotide leader sequence on the mRNA that encodes adenovirus polypeptide IX seems to mediate translation more efficiently than the long tripartite leader that has received so much attention (Lawrence and Jackson, 1982).

[2] The synthesis of polyoma virus T antigen was significantly reduced in only one of the mutants studied by Bendig *et al.* (1980)—a mutant in which the deletion extended to within two nucleotides of the AUG codon. This fits with evidence from other sources that (only) the nucleotides immediately preceding the AUG codon are part of the ribosome recognition sequence.

Secondary structure in viral mRNAs might have various effects on translation.

1. One might expect secondary structure to inhibit more when it occurs near the cap, which is the presumptive entry site for ribosomes, than when a hairpin occurs farther downstream, because 40 S ribosomal subunits once bound must be able to melt secondary structure to some extent. (One knows for sure that 80 S ribosomes melt secondary structure during the elongation phase of protein synthesis; the triplet code could not be read linearly otherwise.) The prediction that 40 S ribosomal subunits can melt their way through secondary structure within the interior of the leader sequence has been verified: introducing a 13-base-pair hairpin (ΔG −30 kcal/mol) 60 nucleotides downstream from the cap did not impair the translation of preproinsulin mRNA *in vivo* (Kozak, 1986b). The effects of secondary structure close to the cap have not yet been tested systematically, but it has been noted that the 5′ end of alfalfa mosaic virus RNA-4 is unfolded (Gehrke *et al.*, 1983) and RNA-4 is a notoriously efficient message. Godefroy-Colburn *et al.*, (1985b) claim more generally that the degree of cap accessibility of the four alfalfa mosaic virus mRNAs correlates with their translational efficiency, but the correlation appears weak. The cap was indeed least accessible on RNA-3, which ranks lowest in translational efficiency, but the cap was equally accessible on RNAs 1, 2, and 4, which differ 15-fold in competitive efficiency (Godefroy-Colburn, 1985a).

2. Although we expect ribosomes to melt secondary structure to some extent, there must be a limit to that ability. Whereas a hairpin of −30 kcal/mol at the midpoint of the leader sequence (involving neither the cap nor the AUG codon) did not reduce the synthesis of preproinsulin under normal culture conditions, a hairpin of −50 kcal/mol nearly abolished translation (Kozak, 1986b). Because the hairpin did not encroach on the AUG codon, the observed inhibition seems incompatible with the direct-binding hypothesis, but is consistent with the scanning hypothesis. Pelletier and Sonenberg (1985) have also shown that translational efficiency decreases as secondary structure in the 5′-noncoding region increases.

3. There is no experimental support for the idea that secondary structure orients the cap and the AUG codon, thus determining which AUG will initiate translation. Were that true, denaturation should impair translation; in fact, denaturation often enhances (Payvar and Schimke, 1979). Nor is there support for the idea that downstream cistrons are silent due to conformational constraints: attempts to activate internal initiation sites by denaturing viral mRNAs invariably fail (Collins *et al.*, 1982; Monckton and Westaway, 1982). A popular

idea is that when secondary structure sequesters the 5′-proximal AUG triplet, it might be skipped by ribosomes in favor of the next exposed AUG codon (Darlix *et al.*, 1982; Ghosh *et al.*, 1978; Hay and Aloni, 1985; Nomoto *et al.*, 1982). The results of a direct test contradict that notion, however. When the primary sequence around the 5′-proximal AUG codon in a chimeric preproinsulin mRNA was favorable for initiation, no translation from a downstream site could be detected *irrespective of whether the first AUG codon was single stranded or base paired* (Kozak, 1986b). Thus, 40 S ribosomal subunits appear to scan linearly, melting the secondary structure ($\Delta G \leq -30$ kcal/mol) to reach each AUG codon in turn. If a hairpin is too stable to be melted ($\Delta G \geq -50$ kcal/mol), the 40 S subunit apparently stalls, but it does not "jump over" the barrier.

4. In some viral mRNAs, sequences at the 3′ end are complementary, to a limited extent, to those at the 5′ end (Antczak *et al.*, 1982; Dasgupta *et al.*, 1980). That arrangement might be expected to inhibit translation—an expectation that has been confirmed recently using mRNAs with artificially constructed terminal complementary sequences (Spena *et al.*, 1985). Some viruses seem to take measures to preclude such inhibition. Whereas the genomic RNAs of influenza (Robertson, 1979) and bunyaviruses (Eshita and Bishop, 1984) have complementary 5′- and 3′-terminal sequences, that potentially deleterious structure is not copied into mRNA, inasmuch as the 3′ terminus of each mRNA stops short of the 5′ end of the template strand (Bouloy *et al.*, 1984; Eshita *et al.*, 1985; Hay *et al.*, 1977). Arenaviruses also produce mRNAs that lack the complementary sequences present at the termini of genomic RNA (Auperin *et al.*, 1984).

5. Incubation in hypertonic culture medium has been used often to study protein synthesis in virus-infected cells (see Yates and Nuss, 1982, and references therein). Hypertonic shock results in the rapid and reversible inhibition of protein synthesis at the level of initiation (Saborio *et al.*, 1974). An intermediate concentration of salt or sucrose permits a residual low level of translation, under which circumstance viral protein synthesis nearly always predominates over cellular protein synthesis (Cherney and Wilhelm, 1979; Garry *et al.*, 1979; Nuss *et al.*, 1975; Oppermann and Koch, 1976). It is difficult to deduce the mechanism of this differential response from inspection of natural forms of viral and cellular mRNAs. However, a cloned preproinsulin gene has been experimentally converted from hypertonic resistant to hypertonic sensitive by inserting into the 5′-noncoding sequence the oligonucleotide AGCTTGGGCCGTGGTGG, thereby creating a 13-base-pair hairpin around the AUG initiator codon (mutant B13hp in Kozak, 1986b). A reasonable interpretation is that the hairpin structure (ΔG −30 kcal/mol), which does not inhibit translation under nor-

mal culture conditions, is stabilized under hypertonic conditions to the point where it becomes inhibitory. An alternative explanation, currently under investigation, is that the primary sequence of the oligonucleotide insert underlies the enhanced sensitivity of mutant B13hp to hypertonic stress. If the first explanation turns out to be correct, one might suggest by extrapolation that most viral mRNAs are less structured near the 5′ end than are most cellular mRNAs, and for that reason viral mRNAs are more resistant to hypertonic stress. Herpes simplex virus mRNAs are a notable exception: they are unusually sensitive to hypertonic inhibition (Stevely and McGrath, 1978), perhaps because their high G + C content generates extensive secondary structure.

6. The mechanism of action of interferon is too complex to discuss here, except to mention that double-stranded regions of RNA, either free or incorporated into the mRNA structure (DeBenedetti and Baglioni, 1984; Knight *et al.*, 1985), are critical in activating and targeting the interferon-induced enzymes. The deleterious effects of interferon on the stability and translation of viral mRNAs have been reviewed by Lengyel (1982).

B. Viral mRNAs Are Usually Monocistronic in Function

The monocistronic rule means more than simply producing one protein from one mRNA. A number of viral mRNAs encode two or more proteins in nonoverlapping reading frames; with few exceptions, however, (see Section II,C), it is exclusively the *5′-proximal cistron* that gets translated (Shih and Kaesberg, 1973; reviewed by Kozak, 1978; and Smith, 1977). To cope with the usual inability of eukaryotic ribosomes to initiate at internal sites in mRNA, the genomes of animal viruses are punctuated at one of four levels, as described below. The structures of plant virus RNA genomes and their patterns of expression have been reviewed by Davies and Hull (1982), and they are not exceptional. The mode of expression of cauliflower mosaic virus, which has a circular DNA genome, is exceptional indeed, and is discussed in Section II,C. The following descriptions are generalized; additional details and references have been published elsewhere (Kozak, 1981b). Each virus is classified according to its major mode of punctuation, which is often not the exclusive mode.

1. *The genome itself is segmented.* Each segment typically consists of one gene, which is transcribed end to end, or nearly so. There is usually a simple correspondence between the size of the mRNA and the size of the mature protein derived therefrom. Reoviruses, influenza viruses, and bunyaviruses fit this description. Arenaviruses and nodaviruses

(e.g., black beetle virus) have segmented RNA genomes but rely also on other mechanisms.

2. The viral genes are linked, but *internal start and stop sites for transcription* generate a separate mRNA for each protein. Punctuation is accomplished for the most part at the level of transcription rather than by posttranscriptional processing. Again, the size of the mRNA usually corresponds to the size of the mature protein.[3] This group includes poxviruses, herpesviruses, rhabdoviruses (VSV), and paramyxoviruses.

3. *Punctuation occurs posttranslationally, by proteolysis.* Here the genome lacks internal transcriptional and translational stop/start sites. The genome-sized mRNA is translated end to end to produce a "polyprotein," more than 2000 amino acids in length, which is cleaved to generate the mature viral proteins. The extreme situation in which all viral proteins are derived from a single precursor is characteristic of picornaviruses and flaviviruses (Castle *et al.*, 1986; C. M. Rice *et al.*, 1985). [Rice *et al.* (1986) present a lucid explanation of some older data that had suggested a different translational strategy for flaviviruses.] Posttranslational cleavage supplements other modes of punctuation in many animal virus systems, and is especially important in the maturation of retrovirus and alphavirus proteins.

4. The fourth, rather heterogeneous group of viruses characteristically produce big transcripts that cannot be translated completely: ribosomes bind at the 5′ end and translate only up to the first stop codon, and the downstream cistrons in these polycistronic mRNAs are usually silent. The downstream cistrons become translatable when they are moved closer to the 5′ end, which is accomplished by producing truncated or *subgenomic mRNAs*. Various mechanisms generate these shortened transcripts. Conventional splicing of nuclear transcripts is used by retroviruses, papovaviruses, and parvoviruses. Adenoviruses also use splicing, on a rather grand scale (Nevins, 1982; Ziff, 1985). Coronaviruses use a novel cytoplasmic fusion mechanism to transfer a common leader sequence to each of six, progressively shorter, subgenomic mRNAs (Budzilowicz *et al.*, 1985; Lai *et al.*, 1984; Spaan *et al.*, 1983). In the case of alphaviruses and parvoviruses, initiation at an

[3] Whereas the molecular weight correlation between mRNAs and proteins holds for most early vaccinia virus genes (Cooper and Moss, 1979; Hruby and Ball, 1982), late vaccinia mRNAs are notoriously heterogeneous in size, apparently because transcription does not terminate discretely (Mahr and Roberts, 1984; Rosel and Moss, 1985). The 3′-proximal portions of such transcripts are assumed to be translationally silent. In the case of herpes simplex virus, the size of many mRNAs corresponds simply to the size of the encoded protein, but more complex mRNAs also exist (Wagner, 1985); the functional significance of the latter is not yet clear.

internal transcriptional promoter produces the subgenomic mRNAs that encode the major capsid proteins (Brzeski and Kennedy, 1978; Janik *et al.*, 1984). Hepadnaviruses (hepatitis B and others) cannot yet be classified, since mRNAs have been identified for some but not all of the viral proteins (Tiollais *et al.*, 1985). The major subgenomic mRNA is initiated at an internal promoter, and there is no evidence for splicing. The heterogeneous initiation sites for transcription in hepatitis viruses might be a means to regulate translation, as suggested by Laub *et al.* (1983) and Enders *et al.* (1985).

Arenaviruses are a special case. The genomic S-RNA segment codes for two structural proteins, N and GPC, but only GPC can be translated conceptually directly from the 5′ half of virion RNA; the 3′ half of the sequence is an antisense version of the N gene (Auperin *et al.*, 1984). Thus, a subgenomic *complementary* mRNA is produced to translate the N protein. Although GPC could in theory be translated from the full-length viral S-RNA, a subgenomic RNA corresponding to the 5′ portion of S-RNA is also present in infected cells. This might be necessary to avoid "hybrid arrest" which could occur if translation were attempted with full-length viral and antiviral transcripts.

C. Mechanisms That Allow Some mRNAs to Direct the Synthesis of More Than One Protein

Whereas most eukaryotic mRNAs are functionally monocistronic, certain viral mRNAs have been shown to synthesize two separately initiated polypeptides. With few exceptions[4] we can rationalize the

[4] The mechanisms outlined herein cannot explain the (inefficient) internal initiation that occurs in a mutant form of Rous sarcoma virus *src* mRNA (Mardon and Varmus, 1983). Poliovirus mRNA also initiates translation at more than one site, at least *in vitro* (Celma and Ehrenfeld, 1975), but one cannot attempt an explanation until the sites have been identified. [Dorner *et al.* (1984) claim to have localized an internal initiation site, but they did not prove that the template RNA was intact. The fact that they could demonstrate "internal initiation" in extracts from reticulocytes but not from poliovirus-infected cells hints of an artifact.] Because the poliovirus 5′-noncoding sequence has eight AUG triplets upstream from the major translational start site (Kitamura *et al.*, 1981; Racaniello and Baltimore, 1981), spurious initiation events are expected in that region. On the other hand, the upstream AUG triplets would not preclude initiation of the polyprotein from the ninth AUG codon, because seven of the upstream AUG triplets lie in a weak context; the only one that lies in a favorable context is followed by an in-frame terminator codon, which would allow reinitiation. The same explanations are compatible with the genomic sequences of many other picornaviruses (Baroudy *et al.*, 1985; Callahan *et al.*, 1985; Forss *et al.*, 1984; Linemeyer *et al.*, 1985). The two structural peculiarities of picornavirus mRNAs—presence of upstream AUG codons and absence of

production of two proteins from a single mRNA by invoking one of the following mechanisms, each of which is experimentally supported. These mechanisms (with the exception of reinitiation) might be considered errors, i.e., the results of imprecise execution of some step in translation. A system that functions with less-than-perfect fidelity apparently gains the advantage of versatility.

1. Leaky Scanning

The scanning model postulates that, when the 5′-proximal AUG codon occurs in a suboptimal context, ribosomes will initiate at that site *as well as at another AUG codon farther downstream.* Several nucleotides near the AUG codon are known to affect the efficiency of initiation, but the most important determinants are a purine (preferably A) in position −3, and G in position +4; we can predict the occurrence of leaky scanning by focusing on those two positions. In each of the bifunctional viral mRNAs listed in Fig. 1, the more 5′-proximal initiation site lies in a suboptimal context, thus rationalizing the ability of some ribosomes to reach the start of the second cistron. (In SV40 16 S mRNA, influenza B, and adenovirus-12, which are bracketed in the center of the figure, the sequence flanking the first AUG codon is not really weak, but it is not perfect; thus, some 10–20% of the 40 S subunits are expected to bypass the first AUG codon and reach the second. That may be adequate to produce the second protein in the case of adenovirus and influenza virus, but it does not seem adequate to explain the synthesis of SV40 VP1, which is an abundant protein. In SV40 16 S mRNA, however, ribosomes can reinitiate at the VP1 start site, as explained below.) The scanning model does not necessitate that the second AUG codon lie in a stronger context than the first, although that usually is the case; it is necessary only that the first AUG codon lie in a context that is less than optimal. Each mRNA listed in the upper part of Fig. 1 produces two unrelated proteins, translated from two different reading frames. The mRNAs in the lower part of the figure initiate at two AUG codons in the same reading frame, thereby producing long and short versions of the same protein.

Whereas the relaxed scanning mechanism accounts qualitatively for the dual function of the mRNAs listed in Fig. 1, the model is not very

---

a cap—might be related: it is possible that, when cap binding protein(s) are not part of the 40 S initiation complex, AUG codons in suboptimal contexts are recognized even less efficiently than usual, and the barrier effect of the upstream AUG codons in poliovirus mRNA would thus be minimized. Perhaps p220 is cleaved (see Section III,D) to directly facilitate viral translation, rather than to inhibit host translation.

good at predicting the frequency with which ribosomes initiate at each site. One problem is that the ratio of initiation at sites 1 and 2 *in vivo* is often different from that *in vitro* (Bos *et al.*, 1981; Clarke *et al.*, 1985; Dethlefsen and Kolakofsky, 1983; Jacobs and Samuel, 1985), and the ratio changes when salt or other reaction conditions are varied. That is hardly surprising because the fidelity of initiation *in vitro* is sensitive to reaction conditions (Jense *et al.*, 1978; Kozak, 1979b; Petersen and Hackett, 1985). On the other hand, the *in vivo* ratio might be skewed if one protein is less stable or less efficiently extracted than the other.

In addition to the obvious economy of using one mRNA to make two proteins, in a fixed ratio, their simultaneous production might allow the polypeptides to interact as the nascent chains grow. It would be amusing to determine whether complementation is less efficient when two proteins that are normally translated from one mRNA are instead synthesized from separate templates.

2. *Initiation at Codons Other Than AUG*

The hundreds of eukaryotic cellular genes that have been sequenced to date invariably initiate translation at AUG. When alternate initiator codons were tested experimentally, however, they were not inert. Eukaryotic ribosomes can initiate at GUG (Kozak, unpublished data) and UUG (Zitomer *et al.*, 1984), but the efficiency is at least 30-fold lower than at an AUG codon in the same context; initiation at GUG, UUG, or other nonstandard codons is (barely) detectable only when the codon is preceded by the optimal A in position −3 (M. K., unpublished data). There is credible, albeit not definitive, evidence that alternate initiator codons are used in two virus systems to produce minor virion components. One is adeno-associated virus capsid protein B, which probably initiates at an ACG codon that lies upstream from the major AUG start site (Becerra *et al.*, 1985). [An ACG codon in coliphage T7 mRNA is also recognized as an initiator codon by wheat germ ribosomes *in vitro* (Anderson and Buzash-Pollert, 1985). Although the template is unnatural in that case, the evidence for initiation at ACG is irrefutable.] The second natural example is $gPr80^{gag}$, a nonessential but nonetheless conserved form of *gag* produced by Moloney murine leukemia virus (Edwards and Fan, 1980; the nucleotide sequence of the region is given by Shinnick *et al.*, 1981). $gPr80^{gag}$ is analogous to the elongated form of *gag* produced by feline leukemia virus, except that the latter is presumably initiated at an upstream AUG codon in a weak context (Fig. 1), whereas in murine leukemia virus the most likely initiation site(s) are upstream GUG and/or CUG codons that lie in a favorable context. Charles Van Beveren has shown that $gPr80^{gag}$ is produced not only by

Sequence of the more 5′-proximal initiation site		BIFUNCTIONAL VIRAL mRNAs	
	C. .AUGG	- - - - Sendai virus (P and C proteins)	TWO PROTEINS TRANSLATED FROM DIFFERENT FRAMES
	C. .AUGG	- - - - Measles virus (P and C proteins)	
	C. .AUGG	- - - - Reovirus types 1 and 3, s1 RNA (σ1, ~14 kd protein)	
	C. .AUGU	- - - - Reovirus type 2, s1 RNA (σ1, ~14 kd protein)	
	G. .AUGU	- - - -[a] Snowshoe hare bunyavirus s-RNA (N, NS_S proteins)	
	U. .AUGG	- - - - Ainobunyavirus s-RNA (N, NS_S proteins)	
	C. .AUGU	- - - - Black beetle virus RNA-3 (B1, B2 proteins)	
	U. .AUGG	- - - - Murine hepatitis coronavirus (12.4 and 10.2 kd)	
	U. .AUGA C. .AUGU	- - - - Infectious bronchitis coronavirus mRNA D (6.7, 7.4, and 1.2 kd proteins)	
	C. .AUGG	- - - - Adenovirus-5 E1B (21 and 55 kd proteins)	
	U. .AUGG	- - - - Adenovirus-7 E1B (21 and 55 kd proteins)	
	U. .AUGA	- - - - Adenovirus-5 E3 (6.7 kd and gp19K)	
see text	G. .AUGG	- - - - Adenovirus-12 E1B (19 and 54 kd proteins)	
see text	A. .AUGA	- - - -[b] Influenza B, RNA-6 (NB and NA proteins)	
see text	G. .AUGG	- - - -[c] SV40 late 16 S mRNA (agno and VP1)	
	U. .AUGG	- - - -[d] SV40 late 19 S mRNA (capsid proteins VP2, VP3)	TRANSLATED FROM THE SAME FRAME
	U. .AUGU	- - - - West Nile flavivirus (V2 core proteins)	
	U. . C. .AUGA A. .	- - - -[e] Foot-and-mouth disease virus (p20a and P16)	
	G. .AUGC	- - -[a,f] Human hepatitis B virus (pre-S, $p24^S$)	
	C. .AUGU	- - - -[g] Feline leukemia virus ($gPr80^{gag}$ and $Pr65^{gag}$)	
	C. .AUGG A. .AUGC	- - - - Herpes simplex thymidine kinase (43, 39, 38 kd)	

FIG. 1. Animal virus mRNAs that direct the synthesis of two separately initiated proteins, which are identified cryptically in parentheses. The left-most column shows that in most cases the sequence around the first functional initiator codon is suboptimal with respect to the nucleotides in positions −3 and +4, thus explaining how some 40 S ribosomal subunits can reach the second initiation site. In the case of the coronaviruses and black beetle virus, the indicated proteins are predicted but have not yet been demonstrated. Although the 6.7-kDa protein predicted from adenovirus region E3 has not been seen, its ribosome binding site has been proven functional by demonstrating the synthesis of a fusion protein from an appropriately engineered mutant virus (Wold *et al.*, 1986). All of the other proteins listed here have been detected in infected cells, and most have also been synthesized in cell-free translation systems. *Notes:* [a]Since the 5′ ends of hepatitis virus and some bunyavirus mRNAs are heterogeneous (Laub *et al.*, 1983; Patterson *et al.*, 1983), the second protein could be translated, without invoking leaky scanning, from the portion of the mRNA population that lacks upstream AUG codons. [b]Influenza virus RNA-6 is unusual in that the first and second AUG codons are separated by only four nucleotides (Shaw *et al.*, 1982), but that probably does not explain the ability of ribosomes to initiate at both sites. In a version of preproinsulin mRNA in which the first and second AUG codons (both in the perfect context for initiation) were

Moloney virus, but also by two other murine leukemia viruses that have no AUG codons upstream from the major ($Pr65^{gag}$) start site (personal communication). Thus, there is no alternative to believing that nonstandard codon(s) are used to initiate the elongated form of *gag*. Experiments to pinpoint the start sites are in progress in Van Beveren's laboratory.

3. *Reinitiation*

Although reinitiation was documented years ago in prokaryotes, there was no reason to suspect a similar phenomenon in eukaryotes until laboratory manipulations with cloned genes yielded some results

---

separated by five nucleotides, ribosomes were unable to initiate at the second member of the pair (Kozak, 1984b). [c]Because the first reading frame terminates upstream from the second in SV40 mRNAs, ribosomes could reach the start site for VP1 by a combination of leaky scanning and reinitiation. [d]The arrangement of AUG codons near the 5′ end of SV40 late 19 S mRNA is GCCAUGG (out-of-frame at position 253–255) . . . UCCAUGG (start of VP2) . . . CCUAUGC (out-of-frame at position 679–681) . . . GGAAUGG (start of VP3) (Reddy *et al.*, 1978). We postulate that leaky scanning allows some 40 S ribosomal subunits to bypass the first AUG triplet (position 253–255) in order to initiate VP2. That does not contradict the fact that, in 16 S mRNA, the AUG codon in position 253–255 initiates the agnogene product. By extrapolating from the systematic measurements carried out in another system (Kozak, 1986a), we would expect 80–90% of the ribosomes to initiate at the AUG codon in position 253–255, while 10–20% should reach the next AUG; that seems sufficient to produce VP2, which is a minor component of the virion. Synthesis of VP3 might depend on leaky scanning (bypassing the first three AUG codons) as well as reinitiation, inasmuch as ribosomes that initiate at the first AUG codon would terminate before reaching the VP3 start site. [e]The nucleotide in position −3 varies among strains of foot-and-mouth disease virus, and the relative yields of P20a and P16 vary accordingly (Clarke *et al.*, 1985). [f]It is likely that two ANNAUG sequences farther upstream are also used to produce longer forms of surface antigen (Heermann *et al.*, 1984). Most transcripts lack the extreme upstream AUG codons, however. [g]The two proteins postulated for feline leukemia virus are indeed seen in infected cells, but the mechanism of synthesis postulated here has not been proven. Infrequent initiation at weak, upstream AUG codons is also suspected with mRNAs from some other retroviruses (Gruss *et al.*, 1981; Willumsen *et al.*, 1984). *References:* Sendai virus: Giorgi *et al.*, 1983. Measles virus: Bellini *et al.*, 1985. Reovirus: Cashdollar *et al.*, 1985; Ernst and Shatkin, 1985; Jacobs and Samuel, 1985; Jacobs *et al.*, 1985; Kozak, 1982; Sarkar *et al.*, 1985. Bunyaviruses: Eshita and Bishop, 1984; Fuller *et al.*, 1983. Black beetle virus: Guarino *et al.*, 1984. Murine hepatitis coronavirus mRNA 5: Skinner *et al.*, 1985. Infectious bronchitis virus: Boursnell *et al.*, 1985. Adenovirus-5 and -12, E1B: Bos *et al.*, 1981. Adenovirus-7 E1B: Dijkema *et al.*, 1982. Adenovirus-5 E3: Wold *et al.*, 1986. Influenza B: Shaw *et al.*, 1983. SV40 16 S mRNA: Jay *et al.*, 1981. SV40 19 S mRNA: Reddy *et al.*, 1978; H. Kasamatsu, personal communication. West Nile flavivirus: Castle *et al.*, 1985. Foot-and-mouth disease virus: Beck *et al.*, 1983; Clarke *et al.*, 1985. Hepatitis virus: Heermann *et al.*, 1984; Pasek *et al.*, 1979; Persing *et al.*, 1985. Feline leukemia virus: Laprevotte *et al.*, 1984. Herpes simplex: Haarr *et al.*, 1985; Wagner *et al.*, 1981.

that are difficult to explain otherwise.[5] The principal observation is that eukaryotic ribosomes can initiate at an internal AUG codon, when another AUG codon occurs upstream and in a highly favorable context (thus ruling out leaky scanning), *provided that a terminator codon occurs in-frame with the first AUG codon and upstream from the second* (Kozak, 1984b; Liu *et al.*, 1984; M. Scott and H. Varmus, personal communication). We envision that when a complete "minicistron," i.e., an AUG triplet followed by a terminator codon, occurs upstream, it is translated; but the 80 S ribosome does not detach at the terminator codon. Rather, the 60 S subunit dissociates while the 40 S subunit remains bound to the message and resumes scanning. When the 40 S subunit reaches the next AUG codon, it reinitiates translation. Reinitiation is more efficient when the terminator codon precedes, rather than when it overlaps, the AUG codon (M. Kozak, unpublished).

With respect to natural mRNAs rather than laboratory constructs, elegant genetic manipulations implicate reinitiation in the translation of Rous sarcoma virus *src* mRNA (Hughes *et al.*, 1984) and cauliflower mosaic virus mRNA (Dixon and Hohn; 1984, Dixon *et al.*, 1986). The latter is the most striking example to date of a functionally polycistronic mRNA in eukaryotes. The overlapping arrangement of cistrons rules out the possibility of reinitiation with many other viral mRNAs (Contreras *et al.*, 1977; Meshi *et al.*, 1983; Schwartz *et al.*, 1983; Skinner and Siddell, 1985). However, in some instances in which adjacent cistrons do not overlap, and reinitiation is therefore expected, it has not been observed (Barker *et al.*, 1983; Goelet *et al.*, 1982; Knowland, 1974; Ou *et al.*, 1982). Reinitiation, together with leaky scanning, could theoretically account for translation of the SV40 agnogene pro-

[5] The alternative to reinitiation is to postulate that eukaryotic ribosomes can initiate directly at an internal AUG codon, and that they usually fail to do so only because the downstream site is occluded by the stream of 80 S ribosomes advancing from upstream. Occlusion indeed occurs during the translation of polycistronic prokaryotic transcripts, but the inhibitory effect of an overlapping upstream cistron is sometimes only two- or threefold (Das and Yanofsky, 1984; Hoess *et al.*, 1980). Berkhaut *et al.* (1985) claimed to see complete inhibition of translation of the MS2 lysis protein when the coat protein cistron overlapped, but the unknown sensitivity of their biological assay complicates the interpretation. Moreover, their claim that a strong upstream initiation site (for coat protein) suppresses initiation from the much weaker site for lysis protein hardly compares with the situation in eukaryotes, where an upstream AUG codon can completely suppress initiation from an *equally favorable* downstream site (Kozak, 1983b, 1984b). The essential difference between the occlusion and reinitiation mechanisms is that the former postulates direct binding of ribosomes to internal AUG codons, while the latter prohibits such binding. There is experimental evidence against direct binding (Kozak, 1979a, 1983b) and against occlusion (Kozak, 1984b).

tein and VP1 from the same mRNA, although neither mechanism has been experimentally demonstrated with SV40. (The simultaneous occurrence of two phenomena complicates the task of demonstrating either one.) Reinitiation is expected within the leader region of Rous sarcoma virus genomic RNA, where three small open reading frames (ORFs), one of them headed by an AUG codon in a highly favorable context, precede the *gag* coding sequence (Schwartz *et al.*, 1983). It has been difficult to demonstrate synthesis of the predicted leader peptides, perhaps because their small size makes them unstable. With admirable persistence, however, Hackett *et al.* (1986) have devised a sensitive assay with which they have detected small amounts of the peptide encoded in the first minicistron of Rous sarcoma virus.

Parenthetically, when one is designing experiments to probe the function of a particular viral or cellular product, one must remember that introducing a nonsense codon near the beginning of a gene might not abolish its function. If an in-frame AUG codon occurs downstream from the nonsense codon, ribosomes will probably reinitiate and the truncated polypeptide might be functional.

4. Frameshifting during Elongation

The mechanism by which reverse transcriptase is synthesized has long puzzled retrovirologists. The *pol* coding sequence is not preceded by an initiator codon; rather, reverse transcriptase is derived by cleavage from a joint *gag–pol* precursor (Murphy *et al.*, 1978; Oppermann *et al.*, 1977). The problem is that the genomic arrangement of *gag* and *pol* sequences would seem to preclude their joint translation. In avian retroviruses, *gag* and *pol* are in different, partially overlapping, reading frames (Schwartz *et al.*, 1983); in murine retroviruses, *gag* and *pol* are in the same frame but are separated by a terminator codon (Shinnick *et al.*, 1981). In both cases, the solution involves a translational "error." With avian retroviruses, about 5% of the ribosomes *shift reading frames* somewhere near the end of the *gag* sequence, thereby producing from one message both *gag* and a small amount of the *gag–pol* fusion protein. Jacks and Varmus (1985) have shown beyond reasonable doubt that frameshifting occurs near the *gag–pol* junction in a cell-free translation system from reticulocytes. By using mRNA that was transcribed *in vitro* from cloned Rous sarcoma virus DNA, they excluded the possibility that a low-abundance, spliced transcript served as the template for the fusion protein. Inspection of the *gag–pol* junction sequences in several other retroviruses leads one to expect that frameshifting is not limited to the avian system. Neither is it limited to eukaryotes, of course. Frameshifting occurs under intrigu-

ing circumstances in a few bacterial and phage genes (Craigen *et al.*, 1985; Dunn and Studier, 1983; Kastelein *et al.*, 1982).

5. Suppression of a Terminator Codon

The excitement that accompanied the old discovery of a "read-through" version of coliphage Qβ coat protein (Weiner and Weber, 1973) has been rekindled recently by finding a similar phenomenon in eukaryotic systems. In murine retroviruses, for example, the *gag* and *pol* sequences are separated by a single UAG terminator codon, the occasional suppression of which generates a *gag–pol* fusion protein. The first hint of this came from supplementing a cell-free translation system with yeast suppressor tRNA, which indeed enhanced the synthesis of the *gag–pol* precursor (Philipson *et al.*, 1978). The notion was confirmed for both murine and feline leukemia viruses when Yoshinaka *et al.* (1985a,b) directly determined the amino acid sequence of the protease that constitutes the NH_2-terminal portion of the *pol* gene product. Suppression of a terminator codon is not peculiar to retroviruses, for it occurs also with alphaviruses (Lopez *et al.*, 1985; Strauss *et al.*, 1983), tobacco mosaic virus (Pelham, 1978), and probably carnation mottle virus (Guilley *et al.*, 1985). Suppression of the UAG codon in tobacco mosaic virus RNA has been traced to the major tyrosine-specific tRNAs which, in tobacco cells, have the anticodon sequence GψA (Beier *et al.*, 1984a,b). The most abundant $tRNA^{Tyr}$ from wheat germ has the highly modified queuine base (Q) in place of G in the wobble position of the anticodon, and it is not able to suppress. Thus, minor differences in tRNA structure can be an important determinant of host range for some viruses.

In one sense, suppression solves the problem of how to produce a full-length protein from an interrupted coding sequence. But that probably misplaces the emphasis. The real problem might be how to produce *only a small amount* of an essential protein that might be toxic if overproduced. An inefficient mechanism, such as suppression or frameshifting, is an ideal solution.

III. The Interplay between Viral and Cellular mRNAs and the Translational Machinery in Infected Cells

Whereas the features described in the preceding section are intrinsic to viral mRNAs, and can be demonstrated readily in a "universal" reticulocyte lysate, the translation of viral mRNAs *in vivo* is influenced by specific conditions that prevail in the cytoplasm of infected cells. The way in which the translational machinery is partitioned

between viral and host mRNAs is one important consideration. Because the literature concerning inhibition of host protein synthesis by animal viruses has already been reviewed at length (Fraenkel-Conrat and Wagner, 1984; Kääriäinen and Ranki, 1984; Shatkin, 1983), I shall be selective in my coverage. An overview of the phenomenology is presented in Table I. The general mechanisms of host shutoff defined by these phenomena are described briefly in Sections B and C, which are followed by a detailed discussion of two viruses—poliovirus and adenovirus—that seem to merit more attention.

The phenomenon of host shutoff is not as widespread as might appear from Table I. Retroviruses, paramyxoviruses, parvoviruses, and flaviviruses do not suppress host translation, and papovaviruses actually stimulate host protein synthesis. Because host shutoff is interesting, and because it is easier to detect viral protein synthesis against a clean background, virologists understandably have focused on systems that demonstrate the phenomenon. The inhibition of host protein synthesis may be of more interest to virologists than to viruses, however. In many cases, the yield of infectious progeny from a virus that fails to shut off host protein synthesis is the same as from another virus strain (or the same virus in a different cell line) in which host protein synthesis is obliterated (Detjen *et al.*, 1982; Gillies and Stollar, 1982; Jen and Thach, 1982; Lodish and Porter, 1981; Minor *et al.*, 1979; Munemitsu and Samuel, 1984; Read and Frenkel, 1983; Sharpe and Fields, 1982). A virus strain that suppresses host macromolecular synthesis sometimes replicates faster in culture than one that does not, however. Whether the inhibition of host protein synthesis is beneficial or harmful or irrelevant to the virus during the course of natural infections is not known. In short, with a few viruses inhibition of host protein synthesis might be a strategic move, necessary for efficient expression of viral genes, but no unequivocal example can be cited. In most instances, host shutoff is likely to be an unintentional side effect of viral gene expression—an effect of no real value, and possibly even harmful, to the virus. It is interesting that poliovirus replicates better during coinfection with cytomegalovirus than during single infection; cytomegalovirus stimulates the cell functions that are turned off by poliovirus (Furukawa *et al.*, 1978)! There are examples of nonpermissive virus–cell systems in which macromolecular synthesis is inhibited so effectively that *neither host nor viral* proteins can be made (Brown and Moyer, 1983; Drillien *et al.*, 1978; Jones *et al.*, 1982). In such cases the wild-type virus must have a way to throttle the shutoff mechanism. That notion will be pursued in the section on adenoviruses.

Throughout this section I have tried to point out wrinkles in the data, uncertainties in some popular interpretations, and alternative

mechanisms. This critical slant is intended not to minimize the value of the work that has been done, but to stimulate reconsideration of some paradigms that may have been accepted or rejected too quickly. Experiments probing the mechanism of host shutoff are difficult. Some of the pitfalls and caveats might be stated at the outset.

A. Cautionary Notes

Certain techniques that are used to block virus infection at a particular step, in order to define the extent of viral expression that is needed to effect host shutoff, might inadvertently create a new inhibitory mechanism. In the resulting confusion one learns little about the physiological mechanism of inhibition. For example, treatment of poliovirus-infected cells with guanidine not only blocks the synthesis of progeny RNA (which is the intended purpose), but also causes double-stranded RNA to accumulate to higher-than-normal levels (Baltimore, 1969); and double-stranded RNA is a potent inhibitor of translation. Experiments showing that a temperature-sensitive mutant virus which makes no progeny RNA nevertheless shuts off host protein synthesis as effectively as wild-type poliovirus suffer the same defect. The mutant-infected cells accumulate massive amounts of partially double-stranded "replicative intermediates" which are likely to inhibit translation, irrespective of the normal shutoff mechanism (Hewlett *et al.*, 1982). In short, the problem with many experiments is that translation can be inhibited in a variety of ways, and in the process of blocking one pathway, another can be activated. For the same reason, the assumption that the mechanism of host shutoff is the same at high multiplicities of infection as at low multiplicities is untenable. In the case of encephalomyocarditis (EMC) virus, the effect on host protein synthesis has been shown to differ qualitatively as a function of multiplicity (Alonso and Carrasco, 1981). With poliovirus, the familiar statement that guanidine does not prevent host shutoff is true *only* when the cells are infected at a high multiplicity (Helentjaris and Ehrenfeld, 1977). At a normal multiplicity of infection, guanidine does block host shutoff, and therefore it is not clear that viral RNA synthesis (which is the guanidine-sensitive step) is uninvolved in the normal mechanism of host shutoff by poliovirus.

The specific deficiency or alteration in the translational machinery can sometimes be pinpointed by studying protein synthesis in extracts prepared from virus-infected cells, provided that one appreciates the limitations of that approach. The notion that one can study the mechanism of host shutoff by one virus by using a second virus as a stand-in for host mRNA is questionable, because proteins encoded by two differ-

TABLE I

CHARACTERISTICS OF THE SHUTOFF OF HOST PROTEIN SYNTHESIS BY ANIMAL VIRUSES

Virus	(1)[a] Timing of host shutoff[b]	(2) Synthesis and transport of host RNA	(3) Integrity of host cytoplasmic mRNA[c]	(4) Overall rate of translation in infected cells	(5) Increased permeability to monovalent cations[d]	(6) Modifications in the translational machinery	(7) Requirements for host shutoff
Poliovirus	Precedes	Synthesis inhibited	Structurally and functionally stable	Rapid decline	Occurs too late	CBP and eIF-2 modified	Translation of input RNA
EMC virus	COINCIDENT	Synthesis inhibited (?)	Functionally stable	Declines ~3 hours postinfection	CORRELATES PERFECTLY	No change in CBP or eIF-2	
Sindbis and SFV	COINCIDENT	Synthesis inhibited	Stable	Declines ~70%	CORRELATES	Weak evidence for change in CBP[e]	Requires viral replication[f]
Vesicular stomatitis virus	COINCIDENT	Synthesis inhibited	Functionally stable	Declines ≥30%	Conflicting data	eIF-2 DEFECTIVE; postulated CBP defect unlikely	
Reovirus	COINCIDENT (type 3)	Synthesis inhibited (type 2 only)	Functionally stable (at least 30%) (type 3)	Varies with serotype, MOI, and cell type[g]	Correlates only with type 3 virus in L cells	~20% PO_4 of eIF-2; weak evidence for altered CBP[h]	σ3 protein; requires viral replication
Influenza virus	COINCIDENT	Synthesis inhibited	Functionally stable but some degradation detectable	No significant change	Not involved	Undefined change postulated, but see text	
Adenovirus	COINCIDENT	Most mRNAs synthesized; not transported[i]	Functionally stable	Early increase then rapid decline		PARTIAL INACTIVATION OF eIF-2	Regions EIB[j] and E4

(continued)

TABLE I (*continued*)

Virus	(1)[a] Timing of host shutoff[b]	(2) Synthesis and transport of host RNA	(3) Integrity of host cytoplasmic mRNA[c]	(4) Overall rate of translation in infected cells	(5) Increased permeability to monovalent cations[d]	(6) Modifications in the translational machinery	(7) Requirements for host shutoff
Vaccinia virus	COINCIDENT	rRNA transport inhibited; mRNA—?	Functionally inactivated and DEGRADED[k]	No change or 50% decline	Occurs too late	No evidence[l]	Requires viral transcription[m]
Herpes simplex virus	Stage 1 precedes; stage 2 coincident	Synthesis inhibited	Stage 1: functionally inactivated; stage 2: DEGRADED	Transient decrease	Not involved	Not studied	Stage 1: virion component[n]; stage 2: needs expression
Frog virus-3	Precedes	Inhibited	Stable			No change in CBP	Virion component

[a]The numbers 1 through 7 in the column headings across the top of the table correspond to the following references, listed by virus. *Poliovirus:* (1) Helentjaris and Ehrenfeld (1978); Nuss *et al.* (1975). (2) Bienz *et al.* (1978). (3) Bossart and Bienz (1981); Fernandez-Munoz and Darnell (1976). (4) Celma and Ehrenfeld (1974). (5) Lacal and Carrasco (1982). (6) Etchison *et al.* (1982); A. Dasgupta, personal communication. (7) Helentjaris and Ehrenfeld (1977). *EMC virus* in HeLa cells: (1) Jen *et al.* (1980). (2) Carrasco and Lacal (1983). (3) Alonso and Carrasco (1981). (4) Jen *et al.* (1980). (5) Alonso and Carrasco (1982b); Lacal and Carrasco (1982). (6) Mosenkis *et al.* (1985); A. P. Rice *et al.* (1985). *Sindbis and SFV:* (1) Lachmi and Kääriäinen (1977); Wengler and Wengler (1976). (2) and (3) Simizu (1984). (4) Van Steeg *et al.* (1981); Wengler and Wengler (1976). (5) Carrasco and Lacal (1983); Garry *et al.* (1979). (6) Van Steeg *et al.* (1981). (7) Simizu (1984). *VSV:* (1) Lodish and Porter (1981); McAllister and Wagner (1976). (2) Grinnell and Wagner (1985). (3) Jaye *et al.* (1982); Lodish and Porter (1980); Nishioka and Silverstein (1978a). (4) Lodish and Porter (1980); Otto and Lucas-Lenard (1980). (5) Francoeur and Stanners (1978); Garry and Waite (1979). (6) Centrella and Lucas-Lenard (1982); Dratewka-Kos *et al.* (1984). *Reovirus* in L cells: (1) Zweerink and Joklik (1970). (2) Sharpe and Fields (1982). (3) Skup *et al.* (1981). (4) Muñoz *et al.* (1985a); Sharpe and Fields (1984). (5) Muñoz *et al.* (1985a). (6) Samuel *et al.* (1984), Skup and Millward (1980). (7) Sharpe and Fields (1982). *Influenza virus:* (1–3) Inglis (1982); Katze and Krug (1984). (4) Lazarowitz *et al.* (1971). (5) Carrasco and Lacal (1983). (6) Katze *et al.* (1984, 1986). *Adenovirus:* (1) Castiglia and Flint (1983). (2) Babich *et al.* (1983); Beltz and Flint (1979). (3) Babich *et al.* (1983). (4) Castiglia and Flint (1983). (6) See text. (7) Babiss and Ginsberg (1984). *Vaccinia virus:* (1) Hruby and Ball (1981); Oppermann and Koch (1976). (2) Salzman *et al.* (1964). (3) Cooper and Moss (1979); Rice and Roberts (1983). (4) Oppermann and Koch (1976); Rice and Roberts (1983). (5) Norrie *et al.* (1982). (7) Bablanian *et al.* (1981). *Herpes simplex:* (1) Pereira *et al.* (1977). (2) Fenwick and Walker (1978); Stenberg and Pizer (1982). (3) Stage 1—see text; stage 2—Inglis (1982); Nishioka and Silverstein (1978b). (4) Silverstein and Engelhardt (1979). (5) Fenwick and Walker (1978); Hackstadt and Mallavia (1982). (7) Fenwick and Walker (1978); Nishioka and Silverstein (1978b); Read and Frenkel (1983). *Frog virus 3:* (6) cited in Mosenkis *et al.* (1985). All other entries are from Willis *et al.* (1985).

[b]The timing of host shutoff relative to the onset of viral translation is indicated. A capitalized entry in this or any other column identifies the probable major mechanism of host shutoff. "Coincident" in capitals means that competition probably underlies host shutoff.

[c]Functional stability is usually evaluated by the ability of host mRNAs, extracted from infected cells, to be translated in a cell-free reticulocyte lysate.

[d]This column indicates the presence or absence of a temporal correlation between the inhibition of host protein synthesis and the influx of sodium ions that often accompanies virus infection (Carrasco and Lacal, 1983).

[e]A change in cap binding protein was postulated because extracts from SFV-infected cells were unable to translate most capped mRNAs, with the exception of EMC and SFV late 26S mRNAs (van Steeg *et al.*, 1981). Although it is true that efficient mRNAs like EMC and SFV 26 S can be translated without benefit of the m^7G cap, it does not follow that cap binding protein(s) are deficient in every instance where translation of those mRNAs persists in the face of an overall decline. Efficient mRNAs will be selectively translated when *any* component of the translational machinery is made limiting. The best evidence for this is the ability of both EMC and SFV 26 S mRNA to be translated in EMC virus-infected cells, in which host translation is drastically inhibited by a mechanism that has not been difined, but that clearly does not involve cap binding protein (Mosenkis *et al.*, 1985).

[f]Van Steeg *et al.* (1984) have postulated that capsid protein is responsible for host shutoff by SFV, but the evidence is not compelling: the binding of host mRNA to ribosomes was only slightly inhibited in Fig. 4 of their paper, and the inhibition was at the level of 80 S rather than 40 S ribosomes. The fact that translation of late viral 26 S mRNA was unaffected is not adequate evidence of specificity, since 26 S mRNA—by virtue of its high efficiency—would be relatively resistant to any inhibitor, physiological or otherwise.

[g]With type-2 reovirus in L cells, infection at a multiplicity of infection (MOI) of 10 caused no significant decrease in translation; at MOI of 20, translation gradually declined by ~40% (Sharpe and Fields, 1982). With type-3 reovirus (MOI of 20), overall protein synthesis was initially stimulated in both HeLa and L cells; translation declined later only in L cells (Muñoz *et al.*, 1985a).

[h]Recent data do not corroborate an earlier hypothesis concerning inactivation of a cap-specific translation factor (Skup and Millward, 1980). Although extracts from reovirus-infected cells translate capped reovirus mRNAs poorly, other cap-dependent mRNAs, such as globin and tobacco mosaic virus, are translated efficiently in such extracts (Lemieux *et al.*, 1984); and capped SV40 mRNAs are translated in cells coinfected with reovirus (Daher and Samuel, 1982). Perhaps translation of capped reovirus mRNAs is inhibited (artificially) in extracts from infected cells because viral structural proteins, which must be abundant in those extracts, adsorb to the homologous mRNAs and sequester them from ribosomes.

[i]In contrast with most other host mRNAs, the synthesis of histone mRNAs is inhibited in adenovirus-infected cells (Flint *et al.*, 1984).

[j]The 55-kDA E1B protein probably functions only indirectly to shut off host translation. The protein is required for efficient cytoplasmic accumulation of late viral mRNAs, which might in turn shut off host protein synthesis by competition (see text). Proteins from regions E1B and E4 may function as a complex.

[k]Host transcripts were stable by hybridization when HeLa cells were infected in the presence of actinomycin D (Rosemond-Hornbeak and Moss, 1975) but were degraded during productive infection of L cells by vaccinia virus (Rice and Roberts, 1983). The second observation seems more pertinent.

[l]Ben-Hamida *et al.* (1983) have purified a component from vaccinia virions that blocks the binding of Met-tRNA to 40 S ribosomes *in vitro*, but the physiological (*in vivo*) mechanism of host shutoff seems to require the expression of viral genes. There is no evidence that eIF-2 function is impaired in infected cells. It is possible, however, that some component in the eIF-2 cycle is altered in a positive way, i.e., a way that prevents inactivation by eIF-2 kinase (Whitaker-Dowling and Youngner, 1984).

[m]It is clear that host translation can be inhibited rapidly in the presence of drugs that preclude the synthesis of viral mRNA (Moss, 1968), but it is not clear that the normal shutoff mechanism is at work in such cases (see text).

[n]The virion-mediated rapid shutoff of host translation is not usually seen with herpes simplex type 1, except in Vero cells; type 2 virus displays the early shutoff function in all cell types. An important, albeit undeciphered, clue is that type 1 virus interferes with the early shutoff by type 2 virions in doubly infected Friend erythroleukemia cells (Hill *et al.*, 1985).

ent viruses can often be produced simultaneously in cells in which host protein synthesis is suppressed (Alonso and Carrasco, 1982a,c; Otto and Lucas-Lenard, 1980). Some features of the intracellular environment, such as ionic changes that favor the translation of viral over host mRNAs, are inevitably lost when the cells are lysed, and other features are not easily preserved. For example, phosphorylated initiation factors have sometimes been inadvertently restored to normal during their purification (Centrella and Lucas-Lenard, 1982; Wong *et al.*, 1982). Phosphorylation of eIF-2 has also been missed on occasion because eIF-2(αP), retains the ability to function stoichiometrically, and the defect is evident only if one assays for catalytic function (Safer, 1983).[6] On other occasions, phosphorylation of eIF-2 has been missed because a high concentration of GTP in the lysate masks the functional defect (Schneider *et al.*, 1985). Extreme care is needed also to preclude the artifactual modification of initiation factors—by proteolysis, for example—during the preparation of cell-free extracts.

The fact that one can reproduce *in vitro* the preferential translation of viral over host mRNAs does not necessarily mean that one is studying the physiological mechanism of host shutoff. If viral mRNAs are even slightly more efficient than host mRNAs, as is often the case, any manipulation that establishes competition will favor the viral mRNAs. One cannot define *how* competition is established *in vivo* by showing *that* competition occurs *in vitro*. For example, the fact that translation of vaccinia mRNAs is more resistant than host mRNAs to inhibition by poly(A) when translation is studied in cell-free extracts from reticulocytes (Bablanian and Banerjee, 1986; Coppola and Bablanian, 1983) does not mean that vaccinia virus inhibits host translation by flooding the cytoplasm with short, polyadenylated transcripts. Such transcripts are indeed produced in infected cells, but only when drugs are used to block the synthesis of normal viral mRNAs (Rosemond-Hornbeak and Moss, 1975). The aforementioned problem of an experimental manipulation creating a new inhibitory mechanism, rather than exposing the normal mechanism, almost certainly applies here.

The tendency to attribute functional significance to foreign agents that cosediment with polysomes should be resisted. Everything cosediments with polysomes to some extent. The presence of a trace of ade-

[6] eIF-2, eukaryotic initiation factor 2, is responsible for binding initiator methionyl-tRNA to the 40 S ribosomal unit, and eIF-2(αP) is eIF-2 phosphorylated on its α-subunit. When eIF-2 is phosphorylated, the reaction in which GDP is exchanged for GTP fails. That reaction is mediated by an accessory protein called GEF, which becomes trapped in an inactive complex with eIF-2(αP). Because the pool size of GEF is small, phosphorylation of only 30% of the eIF-2 pool can completely inhibit translation (Safer, 1983; Siekierka *et al.*, 1984).

novirus VA-RNA in the polysome region of sucrose gradients (Schneider *et al.*, 1984), for example, is almost certainly unrelated to the function of VA-RNA. It is common to find viral capsid proteins stuck to ribosomes, and it is wise to treat such contamination as contamination, until it is proven otherwise.

B. Summary of Mechanisms That Mediate the Switch from Host to Viral Protein Synthesis

The known and suspected mechanisms by which translation of viral mRNAs is facilitated, usually to the disadvantage of host mRNAs, fall into four categories.

1. *Competition* may be suspected when the decline in host protein synthesis and the onset of viral protein synthesis coincide. On the other hand, competition is an insufficient explanation when host protein synthesis is severely inhibited before the onset of viral translation, as occurs with poliovirus, herpes simplex virus, and frog virus 3. Often competition is exacerbated by a decline in the overall translational capacity, which may be brought about by changes in the ionic environment or in the translational machinery. When initiation is limiting, most mRNAs accumulate in small polysomes, the size of which increases upon exposure to a low concentration of cycloheximide. (Cycloheximide slows elongation, thus causing the number of ribosomes to increase on mRNAs that were previously limited at the initiation step.) The characteristic shift in polysome size upon exposure to cycloheximide is seen, for example, in cells infected by VSV (Jaye *et al.*, 1982) or adenovirus (Perlman *et al.*, 1972). The competition between host and viral mRNAs that takes place *in vivo* is sometimes not maintained when the translation of endogenous mRNAs is studied in extracts from infected cells, probably because the concentrations of critical components change during the preparation of such extracts.

2. *Inactivation of a normal component of the translational machinery.* The resulting deficiency enables only a subset of mRNAs, mostly viral, to be translated. The hallmark of this mode of regulation is the ability to restore translation to cell-free extracts by adding back the missing factor. In practice, this is not as easy as it sounds. There is evidence for inactivation of initiation factor eIF-2 in several virus systems, as noted in Table I. Alterations in the initiation factor that mediates the translation of capped mRNAs have been postulated for several viruses (Table I), but the story seems credible only in the case of poliovirus, which is described below.

3. *Production or induction of a dominant inhibitor of translation.* The

identifying characteristic here is that extracts from infected cells cannot be reactivated by the addition of normal initiation factors, but can be reactivated by washing the ribosomes to remove the inhibitor. Based on these criteria, Pensiero and Lucas-Lenard (1985) have postulated the production of an inhibitor during mengovirus infection. Because mengovirus and host mRNAs are equally sensitive to the inhibitor in cell-free extracts, one must invoke competition (for the residual functional ribosomes) to explain the selective persistence of viral translation *in vivo*. This seems justified in view of the extraordinary efficiency of mengovirus mRNA when translation is carried out *in vitro* under conditions of competition (Abreu and Lucas-Lenard, 1976). Until the postulated inhibitor has been identified, however, we cannot be certain that mengovirus belongs in category 3.

4. *Production or induction of a novel factor that specifically promotes the translation of viral mRNAs.* The hallmark here is that viral mRNAs should be translated more efficiently in cell-free extracts from infected than from uninfected cells. Frog virus 3 meets this criterion (Raghow and Granoff, 1983). It is possible that some other animal viruses alter the translational machinery in a "positive" way.[7] The best evidence to date comes from plant viruses, however. A genetic analysis of temperature-sensitive mutants of alfalfa mosaic virus strongly suggests that RNAs-1 and -2 encode or induce a factor that facilitates the translation of coat protein from RNA-4 (Huisman *et al.*, 1985). Extrapolating that mechanism to brome mosaic virus would explain why RNA-4 fails to synthesize coat protein when it is injected (without RNAs-1, -2, and -3) into barley protoplasts (Kiberstis *et al.*, 1981). Unfortunately, cell-free systems from infected plant cells are not available to test the hypothesis. The aforementioned hints are only hints. No virus has yet been proved to produce a new or alter an old translational factor in a way that specifically promotes its own translation.

C. Competition Takes Various Forms

Since competition is the most common mechanism of translational regulation in virus-infected cells, that topic merits more attention. There are at least three variations on the theme.

[7] Although the overall ability to translate poliovirus mRNA is about the same when cell-free systems are reconstituted with factors from infected or uninfected cells (Brown and Ehrenfeld, 1980), there is a qualitative difference in the selection of initiation sites when factors from infected cells are used (Brown and Ehrenfeld, 1979). Other experiments support the idea that poliovirus (Bernstein *et al.*, 1985) as well as vaccinia (Moss and Filler, 1970) and human T-lymphotropic virus type III (Rosen *et al.*, 1986) produce something that enhances the synthesis of viral proteins. The enhancing substance could

1. Competition by mRNA Abundance

In L cells infected by reovirus type 2, host protein synthesis is dramatically shut off. The elegant genetic studies of Sharpe and Fields (1982) revealed that the S4 gene, which encodes the major capsid protein σ3, is responsible for the inhibition. The effect of σ3 might be indirect, inasmuch as the same gene product is responsible for inhibiting host RNA synthesis. Although host shutoff by type 3 reovirus in SC-1 cells is less dramatic than with type 2 virus in L cells, the mechanism of type 3 shutoff is better understood due to the careful quantitative studies of Thach and colleagues (Walden *et al.*, 1981). Their conclusion was rather surprising: the intrinsic translational efficiency of reovirus mRNAs is not higher than that of host mRNAs, but rather, reovirus translation dominates because viral mRNAs accumulate in massive amounts—up to 45% of the total mRNA in the cell! The evidence that reovirus mRNAs initiate translation less efficiently than most host mRNAs is twofold: (1) the size of reovirus polysomes is smaller than host polysomes that code for proteins of comparable size; and (2) whereas a low concentration of cycloheximide reduces the synthesis of host proteins (which is the result expected for mRNAs of "normal" efficiency), the translation of reovirus proteins is actually enhanced by a low concentration of cycloheximide.

Whereas reovirus mRNAs appear to be less efficient than most host mRNAs, VSV mRNAs are probably translated as efficiently as host mRNAs, but not more so. Competition is simply proportional to the concentration of viral mRNAs in the cytoplasm (Lodish and Porter, 1981),[8] and VSV and host mRNAs that encode the same-sized proteins are on polysomes of the same size (Lodish and Porter, 1980). In some cell lines infected by some strains of VSV, a portion of the eIF-2 pool seems to be inactivated (Centrella and Lucas-Lenard, 1982; Dratewka-Kos *et al.*, 1984). Although that would intensify the competition, it is obvious that lowering the eIF-2 level per se cannot explain the selective inhibition of host translation. Selective shutoff requires that viral mRNAs be more abundant than host mRNAs, or more efficient, or

---

be a virus-specific translation factor, or a protease inhibitor that stabilizes viral proteins, or a nuclease inhibitor, or something else. Recent evidence indeed suggests that vaccinia encodes a function that protects late viral mRNAs against degradation (Pacha and Condit, 1985).

[8] The notion that VSV inhibits host translation by competition is not universally accepted, because certain manipulations that prevent viral transcription have been shown *not* to prevent host shutoff. Kääriäinen and Ranki (1984) have reviewed these experiments and have suggested that the mechanism of host shutoff by transcriptionally defective VSV might be unrelated to the normal shutoff mechanism.

both. The aforementioned experiments argue against VSV mRNAs being unusually efficient, but other experiments have been taken as evidence for the contrary view. Because VSV mRNAs are more resistant than host mRNAs to hypertonic stress, Nuss *et al.* (1975) have suggested that the viral mRNAs are intrinsically more efficient. Their interpretation seems reasonable, but it must be carefully circumscribed. If high salt exacerbates some deleterious feature in the mRNA (such as secondary structure) to the point where it *becomes* inhibitory, then the hierarchy of mRNA strengths that one observes under hypertonic conditions might be irrelevant to normal growth conditions.[9]

The progressive inhibition of host protein synthesis during infection by vaccinia virus is probably due to competition, in proportion to the concentration of each mRNA. Viral mRNAs are not apparently more efficient than host mRNAs (Cooper and Moss, 1979; Lemieux *et al.*, 1982). Degradation of host mRNAs (Table I) and the massive synthesis of viral transcripts probably tip the balance in favor of viral protein synthesis. Lemieux and Beaud (1982) have suggested that differential association of mRNAs with the cytoskeleton might also play a role, but that is a difficult hypothesis to test.

2. *Competition by Viral mRNAs That Translate More Efficiently Than Host mRNAs*

In contrast with reovirus and VSV, the concentration of EMC virus mRNA in infected cells may be too low for simple competition to effect the observed switch from host to viral translation, even though EMC mRNA is translated more efficiently than host mRNAs both *in vivo* (Jen *et al.*, 1978) and *in vitro* (Golini *et al.*, 1976; Svitkin *et al.*, 1978). In view of the overall decline in translation that begins 3 hours postinfection, however, the idea that EMC virus mRNA outcompetes host mRNAs for the low, residual translational capacity seems reasonable. The overall decline is most likely due to an influx of monovalent cations, since the two events are temporally correlated (Lacal and Carrasco, 1982). Host translation is restored when EMC virus-infected cells are shifted to hypotonic medium (Alonso and Carrasco, 1981, 1982b), and excess salt, sufficient to inhibit the translation of host mRNAs *in vitro,* dramatically stimulates the translation of EMC RNA (Carrasco and Smith, 1976).

[9]Recall that, although reovirus mRNAs are not more efficient than host mRNAs in unperturbed cells, reovirus translation, like that of VSV, dominates when cells are subjected to hypertonic stress (Nuss *et al.*, 1975). In other studies, the creation of a hairpin (ΔG −30 kcal/mol) within the 5′-noncoding region of preproinsulin mRNA impaired translation only in hypertonic medium; the hairpin did not inhibit translation under normal culture conditions (Kozak, 1986b).

A similar mechanism might mediate the switch from host to viral translation during infection by alphaviruses (see Sindbis and SFV, i.e., Semliki Forest virus, in Table I), since the influx of sodium ions exactly coincides with the overall decline in protein synthesis. The magnitude of the ion influx remains controversial (Gray *et al.*, 1983; Muñoz *et al.*, 1985b). Translation of SFV mRNA is more resistant than host protein synthesis to hypertonic conditions (Garry *et al.*, 1979), but the resistance is not as dramatic as with EMC virus (Alonso and Carrasco, 1982c). The notion that alphaviruses inhibit host translation by competition seems viable even if something more than enhanced permeability to monovalent cations is needed to explain the overall decline. The fact that SFV mRNA can be translated in EMC-infected cells (Alonso and Carrasco, 1982c), in which the overall translational capacity is very low, identifies SFV late 26 S mRNA as an efficient message. Consistent with the competition hypothesis, the time of host shutoff coincides with the production of viral mRNA (Lachmi and Kääriäinen, 1977) and the severity of inhibition correlates with the yield of viral RNA in mutant-infected cells (Atkins, 1976). Polysomes containing SFV (Wengler and Wengler, 1976) or EMC virus mRNA do not increase in size upon exposure to cycloheximide, suggesting that those mRNAs are efficient enough to be fully loaded with ribosomes even when the overall translational capacity is low.

Influenza virus mRNAs are translated with extraordinarily high efficiency *in vitro* (Katze *et al.*, 1986). Because the shutoff of host protein synthesis coincides with the onset of influenza virus protein synthesis and there is no overall decline in translation, simple competition would seem adequate to explain the switch from host to viral translation. In the case of adenovirus, competition is probably exacerbated by a reduction in functional eIF-2 levels. These issues are discussed in more detail in Section III,E.

3. Competition between Early and Late Viral mRNAs

In some virus systems, competition might dictate the switch from synthesis of early to late viral proteins. Picornaviruses, rhabdoviruses, and influenza virus are uninteresting in this regard, as they display little or no temporal control over protein synthesis. The existence of a temporal switch is questionable for reoviruses, but all of the other entries in Table I, as well as the papovaviruses, show a striking early-to-late transition. In every case, the switch is effected primarily at the level of transcription: the mRNAs that encode late proteins are not synthesized until late. In several cases, however, early mRNAs persist in the cytoplasm at late times, and some form of translational regulation seems to limit their expression (Hruby and Ball, 1981; Johnson

and Spear, 1984; Lachmi and Kääriäinen, 1977; Vassef *et al.*, 1982). With black beetle virus that phenomenon can be attributed to competition, because translation of the late mRNA predominates over early mRNA in cell-free extracts under conditions of competition (Friesen and Rueckert, 1984). The same explanation probably holds for alphaviruses. On the other hand, late vaccinia virus mRNAs do not appear to be more efficient than early viral mRNAs (Cooper and Moss, 1979; Oppermann and Koch, 1976). Instead, degradation of some early vaccinia transcripts (Hruby and Ball, 1981) might be part of the switching mechanism. The translation of early mRNAs could be further reduced by the accumulation of "anti-early mRNA" (Boone *et al.*, 1979), which could inhibit translation much as antisense RNA does in other experimental systems (Izant and Weintraub, 1985).[10] With baculoviruses, temporal switching involves the sequential activation of upstream promoters, such that the small, early mRNAs are replaced by progressively longer overlapping transcripts (Friesen and Miller, 1985). The resulting relegation of early protein coding sequences to the 3′ ends of late transcripts probably prohibits their translation. Promoter switching late in SV40 infection also generates forms of mRNA from which T antigen is translated inefficiently.[1] Thus, although transcription plays the dominant role, translational mechanisms—involving competition or other ploys—contribute to the temporal switch in expression of viral genes in some systems.

D. A Closer Look at Poliovirus-Infected Cells

The current thinking is that poliovirus selectively shuts off host protein synthesis by inactivating a 220-kDa protein (p220) which is a subunit of the initiation factor that mediates the translation of capped mRNAs[11] Because the 5′ end of poliovirus mRNA is uncapped, inac-

[10] Few systems other than vaccinia show much potential for regulating translation by "hybrid arrest." Complementary transcripts accumulate in the nuclei of many virus-infected cells, but the complementary sequences are usually edited from cytoplasmic mRNAs. In the case of adenoviruses, for example, where transcription switches periodically from one DNA strand to the other, the 3′ ends of the juxtaposed mature mRNAs rarely overlap (LeMoullec *et al.*, 1983). The 3′ ends of papovavirus early and late mRNAs do characteristically overlap, however.

[11] Although the proteins that can be cross-linked to the m^7G cap have a disturbing tendency to change from year to year, two proteins in mammalian cells that reproducibly cross-link are p24-CBP and p46-CBP. p220 is not a "cap binding protein" inasmuch as it does not cross-link to the cap, but p220 does copurify with p24-CBP and p46-CBP. The aggregate, called eIF-4F, is considered by most people to be the functional "cap binding factor." The functions of cap binding proteins have been reviewed by Shatkin (1985).

tivation of the cap binding factor should not impair the translation of viral mRNA. The idea is appealing because it is straightforward, but some of the supporting data are less so.

The experiment that gave birth to the hypothesis was provocative. Using an antiserum against initiation factor eIF-3, Etchison *et al.* (1982) showed by immunoblotting that p220 is clipped during the first few hours after infection of HeLa cells by poliovirus. Because affinity-purified antibodies against p220 recognized a protein of the same size in some preparations of cap binding factor, the working hypothesis was that cleavage of p220 inactivated the cap-binding initiation factor. Indeed, an activity from uninfected cells that was subsequently purified, based on its ability to restore translation to poliovirus-infected cell-free extracts, contained p220 and the cap binding proteins. These experiments are described below in more detail.

1. Does Cleavage of p220 Mediate the Specific, Early Inhibition of Host Protein Synthesis in Poliovirus-Infected Cells?

This is the most promising explanation to come forth, but there are some irregularities and many lacunae in the supporting data.

1. There is a discrepancy between the kinetics of degradation of p220 and the kinetics of host shutoff (Etchison *et al.*, 1982). The same anomaly occurs during infection by rhinovirus, which degrades p220 in a manner similar to poliovirus (Etchison and Fout, 1985): the rate of translation is still half-maximal at the time when p220 disappears from the polyacrylamide gels. In many of the experiments carried out with cell-free extracts, the question of timing was disregarded, and cells were routinely harvested 3 hours postinfection (Lee and Sonenberg, 1982; Lee *et al.*, 1985a), which is well beyond the point when host translation is precipitously shut off.

2. The extent of cleavage is difficult to evaluate quantitatively. It seems dangerous to accept the recommendation of Bernstein *et al.* (1985) to focus on the accumulation of the 115-kDa cleavage products without also monitoring the disappearance of p220, because cleavage need not always be arrested at the 115-kDa level. In some experiments the concentration of cleavage products in immunoblots from infected cells greatly *exceeded* the concentration of intact p220 in uninfected cells (Bernstein *et al.*, 1985).

3. A p220 cleavage pattern qualitatively similar to that which occurs in infected cells, although not nearly as extensive, is evident in some extracts from uninfected cells (Bernstein *et al.*, 1985; Fig. 5 in Lee *et al.*, 1985a). Because the link between degradation of p220 and virus infection is not tight, it was not surprising to find that the virus-encoded

protease 3C is not responsible for cleaving p220 (Lee *et al.*, 1985b; Lloyd *et al.*, 1985). It would seem wise to include a spectrum of protease inhibitors during the preparation of extracts. Phenylmethylsulfonyl fluoride is the only one routinely used, at concentrations ranging from 5 m*M* (which is adequate; Etchison *et al.*, 1982) to 1 m*M* (Bernstein *et al.*, 1985) or 0.2 m*M* (Lee and Sonenberg, 1982). One is reminded of the old excitement concerning "processing" of SV40 T antigen (Ahmad-Zadeh *et al.*, 1976) which turned out to be an artifact of extraction (Smith *et al.*, 1978).

4. In uninfected HeLa cells, the concentration of p24-CBP is 10-fold lower than p220 (Duncan and Hershey, 1985a,b). [The concentration of p24-CBP is also low in reticulocytes (Hiremath *et al.*, 1985).] If p24 and p220 (together with p46) function as the cap binding complex, large changes in the p220 pool—although easy to detect by immunoanalysis—are unlikely to alter the rate of translation; but small changes in the pool of p24, which might go undetected with immunological or biochemical probes, would significantly impair translation.

5. A mutant of poliovirus called HF121 has been described in which the synthesis of viral RNA is normal in CV-1 cells, but viral protein synthesis is inefficient, host translation is inhibited more slowly than usual, and p220 is not rapidly cleaved (Bernstein *et al.*, 1985).[12] (The phenotype of HF121 in HeLa cells, which are a more natural host than CV-1 monkey cells, is more complex. The synthesis of viral RNA is greatly reduced and *all* protein synthesis, host and viral, is inhibited very early, again without the concomitant cleavage of p220.) The authors argue cogently that wild-type poliovirus appears to encode a function, absent from HF121, that promotes (or "avoids the early inhibition of") viral translation, and they argue less cogently that HF121 is translated poorly as a consequence of the failure to selectively inhibit host translation. To me, the second postulate seems redundant. Lack of the putative positive factor would be sufficient to account for the poor translation of viral mRNA, and the failure to inhibit host translation with normal kinetics could as likely be the result of inefficient viral translation as the cause.[13] If the slow inhibition of translation by

[12] Although cleavage of p220 is not detectable at all in HeLa cells infected by mutant HF121, cleavage products are clearly evident in CV-1 cells at 5 hours postinfection (Fig. 8A, lane 7, in Bernstein *et al.*, 1985). That is later than normal, and the cleavage is less extensive than normal, but some cleavage does occur.

[13] One could argue similarly that cleavage of p220 in wild-type infected cells is the consequence of the abundant accumulation of viral proteins, rather than a precondition. The issue might be resolved by treating wild-type infected cells with guanidine, which allows only limited synthesis of viral proteins while host translation is inhibited with the usual rapid kinetics. It would be informative to know whether p220 undergoes cleavage under those circumstances.

HF121 in CV-1 cells is a delayed version of the normal shutoff mechanism, then cleavage of p220 must not be central to the normal shutoff mechanism. The authors argue, to the contrary, that shutoff by HF121 is mechanistically different, inasmuch as the inhibition affects both host and viral mRNAs in HF121-infected cells, whereas host translation is preferentially inhibited in wild-type-infected cells. However, *selective stimulation* of viral translation (mediated by the product that is defective in HF121) superimposed on a *general inhibition* of translation, would mimic selective inhibition. The authors contend that the ability of guanidine to block host shutoff by HF121 distinguishes it from the normal shutoff mechanism, but the experiment (in CV-1 cells) was done without testing wild-type virus in parallel, as could have been done by adding guanidine at the start of the infection rather than after 3 hours.

6. The assumption that tobacco mosaic virus, Sindbis virus, and VSV mRNAs are appropriate stand-ins for host mRNA in the restoring assay is questionable (Edery *et al.*, 1984; Rose *et al.*, 1978; Tahara *et al.*, 1981). In cells singly infected by VSV, viral mRNAs are translated in preference to host mRNAs under some conditions (Nuss *et al.*, 1975); thus, VSV mRNAs are not equivalent to most host mRNAs. When cells are doubly infected with poliovirus and SFV (which is akin to Sindbis), or with poliovirus and VSV, conditions can be found that allow the simultaneous translation of poliovirus mRNA and the capped mRNAs of VSV or SFV (Alonso and Carrasco, 1982a). Thus, the factor that restores to poliovirus-infected extracts the ability to translate VSV or alphavirus mRNAs might not be sufficient to restore the translation of most host mRNAs. Globin is the only cellular mRNA that has been shown to work in the restoring assay (Edery *et al.*, 1984), and its translational efficiency resembles viral mRNAs more than the average cellular mRNA. It would be reassuring to omit the usual micrococcal nuclease pretreatment of lysates, and show that the addition of cap binding factor to poliovirus-infected cell-free extracts restores the translation of authentic endogenous host mRNAs.

2. *Is p220 an Essential Subunit of the Cap Binding Factor?*

The association of p220 with p24- and p46-CBPs does not prove that p220 is a necessary component of the complex. In early studies, preparations of p24-CBP that lacked the p220 subunit did preferentially stimulate the translation of capped mRNAs in HeLa cell-free extracts (Tahara *et al.*, 1981; Sonenberg *et al.*, 1980). Those results were considered wanting because p24-CBP failed to reproducibly restore translation to extracts from poliovirus-infected cells, whereas an aggregate of p220, p46, and p24 could restore (Tahara *et al.*, 1981). But there is no

reason to reject the aforementioned demonstration that p24-CBP by itself does stimulate in uninfected systems. Using the restoring assay to define the structure of cap binding factor would be acceptable only if one knew that cap binding factor was deficient in infected-cell extracts.

Grifo *et al.* (1983) showed that translation of globin mRNA was stimulated by the p220–p46–p24 aggregate, even when the system was saturated with p24- and p46-CBPs. Those data prove only that the system which they reconstituted from partially purified subfractions of a reticulocyte lysate was deficient in p220; the data do not prove that p220 is an essential component of cap binding factor. (Indeed, translation of the uncapped mRNA from satellite tobacco necrosis virus was stimulated to the same extent as capped globin mRNA.) The function of p220 would be clearer if one could show that antibodies against p220 inhibit the function of cap binding factor. Such experiments have not been reported. Indeed, the immunological evidence is far from convincing even for the original p24-CBP. A monoclonal antibody "directed against cap binding proteins" was shown to inhibit the translation of capped mRNAs, but the antibody reacted with higher molecular weight proteins *and not with p24-CBP* (Sonenberg *et al.*, 1981). The claim that the higher molecular weight polypeptides were related to p24-CBP no longer seems valid, because polyclonal antibodies obtained recently against p24-CBP react only with that polypeptide (Hiremath *et al.*, 1985).

3. *Might Cleavage of p220 Inactivate eIF-3, Rather Than the Cap Binding Factor?*

In extracts from uninfected HeLa cells, p220 copurifies to some extent with both CBPs and eIF-3 (Etchison *et al.*, 1982). Whereas it is known that p220 restores translation to poliovirus-infected extracts when it is introduced in association with cap binding proteins, it is not known whether p220 would also enhance were it introduced in association with eIF-3. In several studies, eIF-3 failed to restore translation to extracts from poliovirus-infected cells, but it was usually tested on an equal weight basis, vis-á-vis the other initiation factors (Table IV in Grifo *et al.*, 1983; Rose *et al.*, 1978). Because eIF-3 is so massive, it must be tested on an equal molar basis.

An experiment which intended to show the eIF-3 from poliovirus-infected cells is fully functional failed to prove the point, because the assay for eIF-3 was carried out in the presence of cap binding factor from uninfected cells (Etchison *et al.*, 1984). The exogenous cap binding factor may have contributed a component (such as p220) which was necessary for, and absent from, infected-cell eIF-3. The assay would

have been more meaningful had an uncapped mRNA been used, thus allowing the function of eIF-3 to be evaluated without the necessity of adding cap binding factor. Whether p220 should be considered a component of eIF-3 or of the cap recognition factor involves more than semantics. Whereas inactivation of cap binding factor would be sufficient to explain the selective inhibition of host translation, eIF-3 is apparently needed for translating all mRNAs; were eIF-3 activity low, poliovirus would have to outcompete host mRNAs for the residual activity.

4. Other Considerations

Casting a wider net might identify other components that are involved in host shutoff by poliovirus. A few candidates have been ruled out. Initiation factors eIF-4A and eIF-4B, for example, appear to be unaltered (Duncan *et al.*, 1983). The normal association of host mRNAs with the cytoskeleton is disrupted shortly after infection by poliovirus (Lenk and Penman, 1979). Whether that is the cause, or the effect, or is unrelated to inhibition of host translation remains unclear. Such a dramatic effect seems unlikely to be gratuitous, but in other systems disruption of the cytoskeleton does not preclude all translation (Welch and Feramisco, 1985). Follow-up studies in the poliovirus system have not significantly extended Penman's original, provocative observation. When Bonneau *et al.* (1985) infected CV-1 cells first with VSV (which does not dissociate host mRNAs from the cytoskeleton) and then superinfected with poliovirus, translation of VSV G and M proteins was inhibited and those mRNAs were released from the cytoskeleton; unfortunately, it was not shown that VSV N and NS mRNAs, which continued to be translated, remained bound to the cytoskeleton. The conclusion that translation requires association with the cytoskeleton hardly seems warranted.

Carrasco has suggested that the increased permeability of virus-infected cells to monovalent cations might mediate the switch from host to viral translation (Carrasco and Lacal, 1983; Carrasco and Smith, 1976). When infected cells are incubated in medium containing sufficient excess NaCl to inhibit the translation of most other proteins, poliovirus translation is fairly resistant (Alonso and Carrasco, 1982a; Nuss *et al.*, 1975), but the resistance is not as striking as with EMC virus (Alonso and Carrasco, 1982b). The stimulation of *in vitro* translation by high salt is also less obvious with poliovirus mRNA than with some other picornaviruses (Bossart and Bienz, 1981). In the natural course of infection by poliovirus, the precipitous decline in host translation occurs within the first 2 hours, prior to the observed increase in intracellular sodium ions (Nair *et al.*, 1979). Moreover, the synthesis of

cellular proteins cannot be reactivated by incubating poliovirus-infected cells in hypotonic medium (Alonso and Carrasco, 1982b), a manipulation that works beautifully with EMC virus. Thus, hypertonicity does not appear to underlie the shutoff of host protein synthesis by poliovirus.

Morrow *et al.* (1985) made the astonishing discovery recently that the host-encoded kinase that is responsible for phosphorylating eIF-2 binds to and mediates the replication of poliovirus RNA. Although that seems about as auspicious as a sheep shaking hands with a wolf, one can think of ways to rationalize such a dangerous move. If the pool of viral RNA that serves as template for replication has to be kept free of ribosomes, for example, the presence of eIF-2 kinase in replication complexes could help by phosphorylating the local pool of eIF-2. Indeed, eIF-2 might become globally phosphorylated in infected cells, and the resulting eIF-2 deficiency could contribute to the inhibition of host protein synthesis. Whereas older studies suggested that eIF-2 was not deficient in polio-infected cells (Brown and Ehrenfeld, 1980; Helentjaris and Ehrenfeld, 1978), the translation of heterologous mRNAs in infected-cell extracts *was restored* to a limited extent by the addition of eIF-2 (Rose *et al.*, 1978)—an effect that the authors chose to ignore. Asim Dasgupta has reopened the question, and his careful measurements reveal extensive phosphorylation of eIF-2(α) in poliovirus-infected cells (personal communication).

E. A Brief Look at Adenovirus-Infected Cells, with Afterthoughts about Influenza Virus

The adenovirus system has generated considerable excitement recently because genetic manipulations, pioneered in Shenk's laboratory, have revealed a regulatory mechanism that is novel, and yet connects to the extensive older literature on inactivation of initiation factor eIF-2. The focal point is a small virus-encoded RNA called VA-RNA_I. Thimmappaya *et al.* (1982) found that, in cells infected by an adenovirus mutant that produced no VA-RNA_I, late viral mRNAs were synthesized, processed, and transported, but failed to be translated. In the absence of VA-RNA_I, translation was blocked at the level of initiation (Schneider *et al.*, 1984) and the defect was ultimately localized to eIF-2. Overwhelming evidence now supports the hypothesis that, in the absence of VA-RNA_I, a kinase becomes activated that phosphorylates, and thus inactivates, eIF-2 (Reichel *et al.*, 1985; Schneider *et al.*, 1985; Siekierka *et al.*, 1985). eIF-2 kinase (one of the enzymes involved in the antiviral action of interferon; see Lengyel,

1982) exists in uninfected HeLa cells in an inactive state, and is apparently activated by double-stranded RNA that accumulates in infected cells as a by-product of adenovirus transcription (O'Malley *et al.*, 1986).[14] The exact mechanism by which VA-RNA$_I$ blocks the action of eIF-2 kinase is not yet known. An intriguing scenario can be extrapolated from a model that was proposed by Rosen *et al.* (1981) in another context. Their model proposes that high molecular weight double-stranded RNA activates *and targets* eIF-2 kinase: because both the kinase and eIF-2 have binding sites for dsRNA, high molecular weight dsRNA could link the two proteins.[15] By virtue of its small size, VA-RNA$_I$ might be able to bind to eIF-2 or to eIF-2 kinase, but not simultaneously to both. VA-RNA$_I$ would thus block the phosphorylation of eIF-2 much as a monovalent hapten blocks antigen–antibody interactions. The proposal rationalizes the known properties of VA-RNA$_I$: its small size (about 160 nucleotides), doubled-stranded structure (Monstein and Philipson, 1981), and the high concentration that is required to confer protection. Whether the double-stranded regions of VA-RNA$_I$ are crucial for its function is not yet clear. Bhat *et al.* (1985) have mutated VA-RNA$_I$ and found that extensive regions could be deleted without affecting biological activity, although certain other mutations were deleterious. Further experiments will be needed to pinpoint the essential region(s) in VA-RNA$_I$. A second adenovirus-encoded species called VA-RNA$_{II}$ rescues translation far less efficiently than VA-RNA$_I$ (Thimmappaya *et al.*, 1982), and VA-RNA$_{II}$ appears less extensively base paired (Mathews and Grodzicker, 1981).

In addition to its proven protective effect on eIF-2, it has been suggested that VA-RNA$_I$ might interact directly with viral mRNAs to promote their translation (Kaufman, 1985; Schneider *et al.*, 1984; Svennson and Akusjarvi, 1985). A sequence-specific interaction seems unlikely, however, because the small RNAs encoded by Epstein–Barr virus (which are related to adenovirus VA-RNAs by size but not sequence) can substitute to some extent for VA-RNA$_I$ (Bhat and Thimmappaya, 1983, 1985), and the facilitating effect of VA-RNA$_I$ extends

[14] A virus that is protected (by VA-RNA or some other mechanism) from the deleterious effects of its own symmetrical transcription process would also have some resistance to interferon. An interesting story along these lines is emerging with vaccinia virus (Rice and Kerr, 1984; Whitaker-Dowling and Youngner, 1984).

[15] Sen *et al.* (1978) showed that, once kinase has been activated by binding dsRNA, incubation with ribonuclease III does not abolish the ability of kinase to phosphorylate histone H1 (which was chosen as a convenient substrate), but neither the extent of trimming by the nuclease, nor the activity of the trimmed kinase on eIF-2, were determined. Thus, the experiment does not contradict the targeting hypothesis for dsRNA.

not only to late adenovirus mRNAs that carry the standard tripartite leader, but also to early adenovirus mRNAs and late mRNAs with a truncated version of the tripartite leader (Svensson and Akusjarvi, 1984), adeno-associated virus mRNAs (Janik *et al.*, 1982), and various heterologous mRNAs (Svensson and Akusjarvi, 1985).

The protective effect of VA-RNA$_I$ and the shutoff of host translation in adenovirus-infected cells might be two aspects of a single mechanism. If one postulates that the production of VA-RNA$_I$ by wild-type adenovirus is sufficient to protect only a portion of the eIF-2 pool, the switch from host to viral protein synthesis could occur because late adenovirus mRNAs outcompete host mRNAs for the small residual translational capacity. The hypothesis that competition occurs during the late stage of infection by adenovirus is not entirely ad hoc. The overall translational capacity is low late in the infection (Castiglia and Flint, 1983); a portion of the eIF-2 pool is phosphorylated (M. Mathews, personal communication); polysomes are small and their size increases in response to a low dose of cycloheximide (Perlman *et al.*, 1972); and the decline in host translation correlates with the temporal onset and magnitude of late viral translation (Castiglia and Flint, 1983). Every mutation that has been shown to prevent host shutoff also prevents the cytoplasmic accumulation of late viral mRNAs (Babiss *et al.*, 1985; Halbert *et al.*, 1985). An interesting set of experiments by Logan and Shenk (1984) can be rationalized in terms of competition during the late stage of infection. They observed that transposition of the late tripartite leader to the early E1A genes had no effect on the efficiency of translation of E1A products at early times, but significantly enhanced the translation of E1A mRNAs at late times. This is understandable if there is no competition early in the infection, allowing efficient and inefficient mRNAs to be translated equally well. The facilitating effect of the tripartite leader would become evident only late in the infection, when eIF-2 has been partially inactivated and competition has set in.

One might think along similar lines to explain the surprising ability of influenza virus mRNAs to be translated in adenovirus-infected cells (Katze *et al.*, 1984). In wild-type adenovirus-infected cells, in which host protein synthesis is drastically reduced, both adenovirus and influenza virus mRNAs are translated efficiently. In cells infected by dl331, a deletion mutant that produces no VA-RNA$_I$, adenovirus proteins are translated inefficiently, as noted above, but influenza virus proteins are still synthesized in abundance. Despite that striking observation, there is little support or necessity for the notion that influenza virus establishes its own translational system. A simpler explanation is that the very low capacity for protein synthesis that persists in the absence of VA-RNA$_I$ is sufficient for the translation of influenza virus

mRNAs.[16] For that explanation to be correct, influenza virus mRNAs would have to be translated with extraordinary efficiency, and that prediction has recently been confirmed (Katze *et al.*, 1986). What makes all this so intriguing is that the 5′ ends of influenza virus mRNAs, which presumably dictate their high translational efficiency, are derived from host mRNAs (Krug, 1981). It appears as if the viral cap-specific endonuclease (which selects the cellular mRNAs that will serve as donors) is biased toward the same features that facilitate translation. Indeed, that deduction has been verified directly, at least *in vitro*. Bouloy *et al.* (1978, 1980) found that β-globin mRNA, which is translated more efficiently than α-globin mRNA, is also a more efficient primer for influenza transcription; and alfalfa mosaic virus RNA-4, which translates *in vitro* with extraordinary efficiency, is the best known primer for influenza transcription. [From the fact that mRNAs with 2′-*O*-methyl groups in the penultimate position of the cap function better than monomethylated caps as primers for influenza virus transcription (Bouloy *et al.*, 1980), one is tempted to suggest that 2′-*O*-methyl groups enhance translation, although there is little direct evidence for that view!] If the selection of primers in infected cells follows the pattern that is seen *in vitro*, the influenza virus takeover scheme is indeed remarkable: the most efficient cellular mRNAs would be sacrificed to construct viral mRNAs that *ipso facto* translate most efficiently.

IV. Late Night Thoughts

A few other possibilities for regulating translation in virus-infected cells are discussed briefly below. None of these topics is well understood at present, and the musings should be considered little more than that.

A. Possible Implications of Reinitiation and Related Phenomena

Cauliflower mosaic virus seems to be the only case in which the ability of eukaryotic ribosomes to reinitiate is fully exploited to produce several full-length proteins from one mRNA. The structure of a few

[16] Katze *et al.* (1986) have shown that influenza virus partially suppresses the activation of eIF-2 kinase, and they suggest that this underlies the ability of influenza virus to replicate in cells infected by adenovirus mutant dl331. That interesting hypothesis would be stronger if it could be shown that influenza virus replicates even in cells infected by the adenovirus double mutant ($VA_I^-VA_{II}^-$), and if superinfection with influenza virus could be shown not just to reduce the level of activated kinase, but to increase the level of functional eIF-2.

other viral and cellular mRNAs leads one to predict that reinitiation is necessary for ribosomes to reach the major protein coding sequence, but the upstream open reading frames (ORFs) in such mRNAs are characteristically short. In some cases, however, the small peptides encoded near the 5′ end of the message might be biologically important. Genetic studies indicate that this is certainly the case with the agnogene product of SV40 (Margolskee and Nathans, 1983). In contrast, the three peptides encoded within the avian retrovirus leader sequence probably are not functional because there is little conservation of amino acid sequences among virus strains (Hackett *et al.*, 1986). In retrovirus mutants that lack most of the leader sequence, the only known deficiency is the absence of a cis-acting packaging signal (Mann *et al.*, 1983; Nishizawa *et al.*, 1985). Comparison of different strains of poliovirus reveals that the number of upstream AUG codons varies and the coding properties of the small ORFs are not conserved (Toyoda *et al.*, 1984).

Upstream minicistrons that do not encode anything interesting might nevertheless be important for regulation. Several possibilities come to mind for retroviruses. The least interesting idea is that upstream AUG codons accumulate, not from design, but from default—because the deleterious effects on translation can easily be compensated by using efficient transcription signals to mass-produce retrovirus mRNAs. The opposite view is that upstream AUG codons are deliberately retained to throttle the synthesis of a protein that would be harmful if overproduced (Tarpley and Temin, 1984). While that seems a reasonable ploy to use for oncogenes, it makes little sense when extended to viral structural genes. A third possibility derives from the observation that reinitiation usually is not 100% efficient. With preproinsulin constructs, for example, in which the efficiency of reinitiation is routinely 20% (Kozak, 1984b), one might ask what the remaining 80% of the ribosomes are up to. One scenario is that, after 80 S ribosomes have moved through the 5′-proximal ORF, 80% of the 40 S subunits detach at the terminator codon while the rest remain on the message, resume scanning, and reinitiate at the second AUG codon. A more interesting possibility is that all 40 S subunits remain bound and resume scanning, but only 20% reinitiate at the closest AUG codon, perhaps because the codon-recognition step in inefficient in the absence of met-tRNA$_i$, cap binding proteins, and/or other initiation factors—all of which were presumably released at an earlier step. [We do not know the precise sequence of events during initiation, but it seems likely that the factors that mediate the binding of met-tRNA$_i$ and mRNA to the 40 S ribosomal subunit are released prior to or during the joining of the 60 S subunit at the first AUG codon (Moldave, 1985).] If the factor-deficient 40 S subunits that are unable to reinitiate at the second AUG

codon eventually become competent, they might reinitiate father downstream. Thus, the effect of an upstream minicistron could be to loosen the process of initiation in a way that permits ribosomes to reach otherwise inaccessible internal AUG codons. There is no evidence for this, as yet. We know only that reinitiation at the closest AUG codon (following a terminator codon) is less than 100% efficient.

Yet another way in which ribosomes might gain access to internal AUG codons, even in a message in which the major open reading frame initiates with a "strong" AUG codon, relies on the presence of weak, out-of-frame initiator codons in the retrovirus leader sequence and the ability of ribosomes to reinitiate. This hypothetical scheme is best illustrated by using as an example an avian retrovirus mRNA that encodes the *env* glycoprotein (Fig. 2). Katz *et al.* (1986) have studied the effects of mutations in the leader region of this mRNA, using as an

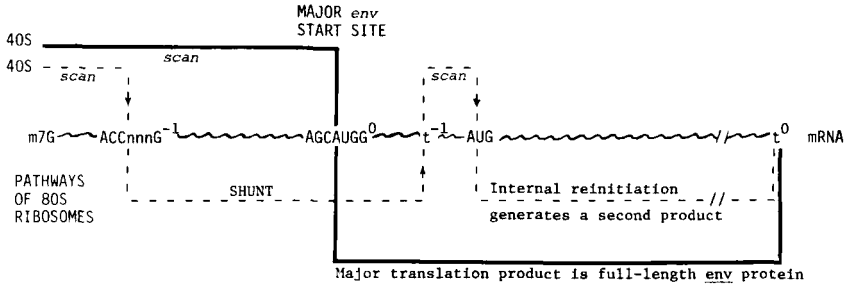

FIG. 2. A hypothesis whereby minor initiation sites in the leader sequence of retroviruses create a shunt that directs ribosomes to internal initiation sites. The diagram represents a subgenomic mRNA that encodes the *env* protein of avian leukosis virus (Katz *et al.*, 1986). Messenger RNA is represented by a wavy line, the pathway followed by 40 S ribosomal subunits is shown above the mRNA, and the pathway of 80 S ribosomes is shown below the mRNA. A solid black line traces the pathway followed by most 40 S subunits: they scan from the m^7G cap to the start of the *env* coding sequence, marked "major start site," where a 60 S subunit joins and translation begins. (Some 40 S subunits will stop and initiate at three upstream AUG codons, but in each instance there is a nearby terminator codon, enabling ribosomes to reinitiate. Thus, the upstream AUG codons are irrelevant for the present discussion and are not shown.) Of more significance are the many *nonstandard* codons (GUG, UUG, etc.) that lie in the *standard* context for initiation. Such codons occur frequently in the −1 reading frame which is open (in the functional EV-2 viral genome) over a stretch of about 200 nucleotides preceding the major *env* start site; the open −1 reading frame ends 125 nucleotides beyond the start of the *env* coding sequence at $UAA_{5188-5190}$, which is labeled t^{-1} in the figure. Were a few 40 S subunits to recognize the nonstandard upstream codons as initiation sites, the resulting 80 S ribosomes—translating in the − frame—would bypass the normal *env* start site. A dashed line traces the pathway of this shunt. The main point is that ribosomes that terminate at t^{-1} could reinitiate at an internal site which would be inaccessible were it not for the shunt.

assay the ability to complement a replication-defective (env$^-$) strain of Rous sarcoma virus. Their results are provocative. Point mutations in positions -4 and -7 (i.e., 4 and 7 nucleotides upstream from the AUG codon that initiates *env*) caused a 10-fold reduction in complementation. On the other hand, the translational efficiency of deletion mutants varied from 5 to 106% of the wild type level, and the variation did not correlate with the presence or absence of any particular portion of the leader sequence. To explain this puzzling pattern (or rather the absence thereof), Skalka suggests that the mutations perturb some aspect of secondary structure that is critical for translation. Because that idea is difficult to formulate in a way that can be tested, it can do no harm to consider an alternative explanation. The biological assay that was used has the advantage of being exquisitely sensitive, but it has the disadvantage of measuring the yield of *env* protein only indirectly: the authors did not show a 10-fold reduction in *env* synthesis; they showed a 10-fold drop in complementation. What if complementation were to require, in addition to *env*, a second minor protein—either a truncated form of *env* that initiates a little farther downstream or a small protein encoded in an alternate reading frame? (There is an open reading frame beginning at $AUG_{5811-5813}$, for example, that could direct the synthesis of a 10-kDa protein.) Because the context at the major *env* start site is highly favorable, all of 40 S ribosomal subunits that reach that site should initiate there; production of the putative internally initiated protein would therefore require a mechanism for shunting some ribosomes beyond the major *env* start site. The hypothesis illustrated in Fig. 2 is that a small fraction of the ribosomes initiate within the leader sequence at weak sites (*nonstandard codons* that lie in a favorable context for initiation) in the -1 reading frame, and translate in that frame past the major *env* start site, terminating at the site labeled t^{-1} in Fig. 2. The small fraction of ribosomes that follow this shunt could reinitiate to produce the second protein postulated above. The notion that the *env* gene encodes two products is certainly ad hoc, but it rationalizes the behavior of Skalka's mutants. The deleterious mutation in position -4 creates a terminator codon in the -1 reading frame, which would short-circuit the shunt and prevent synthesis of the internally initiated protein. In all of the deletion mutants that fail to complement efficiently, the weak upstream start sites are either in-frame with the major *env* start site or terminate upstream from it—again abolishing the shunt. On the other hand, all of the deletion mutants that retain the ability to complement efficiently retain one or more weak upstream start sites (such as GUG in position 132–134 in mutant 137/349, or UUG in position 22–24 in mutant 65/349) which can feed ribosomes into the shunt. The hypoth-

esis could be tested in two ways. One is to directly measure the yield of the major *env* protein—which we predict will not vary, because it is the internally initiated protein that is deficient in these mutants. The best test would make use of a null mutant called pd99/394 that lacks the major *env* start site: that mutant should still make the second protein encoded within the *env* gene, and therefore should complement all of the other mutants that have lost the shunt.

B. Possible Coupling of Transcription, Transport, and Translation

Viruses that replicate in the cytoplasm have the potential for coupling transcription with translation. For example, if ribosomes were to bind the 5′ end of reovirus mRNAs as the nascent chains emerge from the subviral particle, the mRNAs would be recruited for translation before the chains grew long enough to fold. That might enhance translation considerably, because the pattern of cleavage by T_1 RNase suggests that the capped terminus might be sequestered in mature reovirus mRNAs (Kozak and Shatkin, 1978b). It would be fun to know whether reovirus mRNAs are translated more efficiently in naturally infected cells than in cells transfected with cloned viral genes which are transcribed from a plasmid vector. The idea of coupling is ad hoc for reovirus, but there is a glimmer of evidence in the case of silkworm cytoplasmic polyhedrosis virus; whereas performed viral mRNAs were inactive in reticulocyte or wheat germ translation systems, viral proteins were synthesized during coupled transcription–translation in frog oocytes (Ikegami *et al.*, 1985). Payne and Mertens (1983) obtained somewhat different results, in that some viral proteins were made in vitro in the absence of transcription; but the polyhedron protein that predominates in vivo was still not produced in vitro. In the vaccinia virus system, Cooper and Moss (1978) observed more efficient synthesis of vaccinia proteins when transcription and translation were coupled.

Synergism could also occur in the opposite direction; i.e., viral transcription might be facilitated by translation. During the early hours of reovirus infection in L cells, transcription is mainly from genome segments M3, S3, S4, and one of the L segments (Nonoyama *et al.*, 1974). Because mRNAs from segments M3, S3, and S4 bind ribosomes very efficiently (Shatkin and Kozak, 1983), one wonders whether preferential transcription is the consequence of preferential translation.[17] The

[17] The hypothesis is complicated, but not necessarily contradicted, by the finding that M3, S3, and S4 are preferentially transcribed *in vivo* even in the presence of cycloheximide (Shatkin and Kozak, 1983). Although cycloheximide blocks elongation by 80 S ribosomes, 40 S ribosomal subunits could still bind to the nascent transcripts.

possibility that coupled translation enhances transcription was fleetingly entertained for some other cytoplasmic viruses (Ball and White, 1978; Cooper and Moss, 1978), but the reticulocyte lysate appeared to enhance transcription only because it conferred protection against nucleolytic degradation (Pelham *et al.*, 1978). It remains possible that transcription and translation are obligatorily coupled in some less well studied RNA viruses, as has been hinted for bunyaviruses (Patterson and Kolakofsky, 1984; Pattnaik and Abraham, 1983).

In the case of viruses that replicate in the nucleus, the possibility that movement of mRNAs out of the nucleus might be coupled with translation has been raised from time to time. Coupling clearly is not obligatory, because viral mRNAs accumulate in the cytoplasm under many circumstances in which translation is blocked. A good example is the cytoplasmic accumulation of late adenovirus mRNAs in the absence of VA-RNA$_I$. On the other hand, the transport and translation of mRNAs are sometimes coordinated. A striking example occurs in adenovirus-infected HeLa cells that are superinfected with influenza virus (Katze *et al.*, 1984): whereas adenovirus blocks both the transport and translation of host mRNAs, influenza virus mRNAs escape *both* blocks. The probable mechanism that enables influenza virus mRNAs to be translated was discussed in Section III,E. What mechanism enables influenza mRNAs to bypass the block that retains host mRNAs in the nucleus? Katze *et al.* (1984) suggested one possibility, namely, an influenza virus-specific transport system. But it seems simpler to look for a single explanation that would account for both the preferential transport and translation. There could be competition at the level of transport, and the same features that make a message highly translatable might make it highly transportable. An alternative view is that the two processes are coupled. One might envision 40 S ribosomal subunits monitoring the nuclear pores, such that only mRNAs that can be translated under given circumstances will be transported. Along those lines, Babiss *et al.* (1985) have noted that, whereas host mRNAs are neither transported nor translated in wild-type adenovirus-infected cells, transport and translation of host mRNAs are coordinately restored by mutations in early viral genes that reduce the cytoplasmic accumulation of late viral mRNAs. As an extension of the idea that a message will be transported only if it can be translated, one might suggest that mRNAs are transported as soon as they become translatable. The consequence would be that translation could sometimes regulate the extent of splicing. Some splicing events that could occur, were the transcript kept longer in the nucleus, would be prevented by "prematurely" pulling the mRNA out. Svensson *et al.* (1983) invoked this notion to explain some of their observations

on the processing of adenovirus early mRNAs. Coupling of splicing with transport, and transport with translation, would explain why few if any incompletely processed transcripts enter the cytoplasm: no matter how many introns are present in a primary transcript, it remains in the nucleus until every intron has been removed—in effect, until it becomes translatable. It would seem as if the easiest way to judge whether a transcript is translatable is to attempt to translate it.

C. Destructive Modes of Regulation

The shutoff of host protein synthesis by herpes simplex virus might not involve a modification in the translational machinery per se. Late (second-stage) shutoff is clearly caused by the massive degradation of host mRNA. The puzzle of how the nuclease is targeted, such that it degrades host but not viral mRNAs, has not yet been solved. A partial explanation might be that herpes virus mRNAs are more highly structured, by virtue of their high G + C content. The unusual sensitivity of herpes virus mRNAs to hypertonic stress is consistent with the hypothesis that they have extensive secondary structure. The irreversible (Read and Frenkel, 1983) early shutoff of host translation by a structural component of the herpes virion also seems to involve cleavage of host mRNAs—enough to inactivate them for translation (Fenwick and McMenamin, 1984), although they can still be detected by hybridization, more or less (Nishioka and Silverstein, 1978b; Schek and Bachenheimer, 1985). Since mutants that are defective in stage-one shutoff can still induce secondary shutoff of host protein synthesis (Read and Frenkel, 1983), two distinct viral gene products, either nucleases or activators thereof, are apparently involved. A herpes virus mutant that is defective in stage-one host shutoff is defective in switching off the translation of early viral mRNAs as well (Read and Frenkel, 1983). The differential accumulation of adenovirus early mRNAs is also mediated, in part, by the regulated degradation of some transcripts (Wilson and Darnell, 1981). Degradation of host mRNAs might be part of the mechanism by which vaccinia and influenza viruses reduce host translation (see Table I), although clear-cut genetic evidence, such as that described for herpes virus, is lacking in those systems.

The extent to which gene expression is regulated by posttranslational proteolytic degradation is probably not fully appreciated. There are striking, isolated examples, for example, the selective degradation of measles virus M protein (Sheppard *et al.*, 1985), the rapid turnover of some early adenovirus proteins (Spindler and Berk, 1984b), and stabilization of the cellular protein p53 by its interaction with SV40 T

antigen (Oren *et al.*, 1981). Given the intricacies of the ubiquitin pathway for proteolysis, it might be surprising were that pathway not perturbed by virus infection. Some animal viruses might encode a function that protects foreign (i.e., viral) proteins from degradation, analogous to the *pin* function of bacteriophage T4 (Simon *et al.*, 1983).

D. Codon Usage

Although the pattern of codon usage in viral genes is sometimes different from that of the cellular genome, imbalances in the tRNA pool probably do not affect the yield of most viral proteins because the rate-limiting step is usually initiation rather than elongation. Moreover, while there is convincing evidence for a preferred pattern of codon usage in highly expressed bacterial and yeast genes (Bennetzen and Hall, 1982; Ikemura, 1981, 1982), codon preference seems to be more relaxed in higher eukaryotes (Tso *et al.*, 1985), and therefore the cellular tRNA pool might not be markedly skewed. Consistent with the idea that codon usage is not a major regulatory factor in viral gene expression, the close conservation of amino acid sequences between some viruses is not always accompanied by conservation in the choice of codons (Ou *et al.*, 1982).

The degree to which expression might be limited by tRNA deficiencies has been tested in *Escherichia coli* by using cloned genes that are rich in rare codons. The availability of tRNA was found to limit translation only when the mRNA concentration was extraordinarily high (Pedersen, 1984; Robinson *et al.*, 1984). Codon usage might regulate translation in more subtle ways, however. One possibility with some experimental justification is that ribosomes pause briefly at rare codons (Lizardi *et al.*, 1979; Misra and Reeves, 1985; Varenne *et al.*, 1984).[18] Discontinuous elongation is not incompatible with efficient translation, as pausing has been detected during the synthesis of some very abundant proteins (Cepko and Sharp, 1982; Lizardi *et al.*, 1979). Slowing translation in certain positions might facilitate folding of the polypeptide and/or its interaction with other components, however. The pattern of codon usage in the signal peptide portion of some genes encourages this notion (Spieth *et al.*, 1985). The suppression of nonsense codons and the occurrence of frameshifting (see Section II,C) might also be facilitated by an imbalance between the cellular tRNA pool and the viral pattern of codon usage.

[18] An alternative explanation for discontinuous elongation is that ribosomes pause when they encounter hairpin structures in the mRNA, but that idea is without experimental support.

V. Concluding Remarks

What we have learned about the structure and function of animal virus mRNAs can often be extrapolated to cellular mRNAs. The mechanism of selecting the initiation site for protein synthesis certainly appears to follow a single formula. The translational machinery displays a certain flexibility (leaky scanning, frameshifting, etc.) that is exploited more frequently by viral than by cellular mRNAs. That no doubt reflects the limited coding capacity of most viral genomes. In contrast, it would seem easier and more efficient for the expansive cellular genome to separately encode two versions of a protein than to attempt to skirt the "monocistronic rule" in the ways described for viruses.[19] It is important to remember that there are rules for breaking the monocistronic rule. Using those principles, we can correctly predict the qualitative aspects of viral protein synthesis, with very few exceptions. We understand much less about the quantitative aspects of translation, however. Although some of the parameters that determine efficiency have been identified in the preceding pages, or at least surmised, we usually cannot predict how efficiently a given mRNA will be translated by summing the known parameters. Future studies will almost certainly uncover other features that affect translational efficiency: "repressor" proteins, perhaps, or helix-unwinding proteins, or effects of 3′-noncoding sequences, or aspects of mRNA primary and secondary structure that are not yet obvious. The suggestion that it is easier to block translation than to enhance it merits repetition. The most efficient mRNAs might be those that *cannot* interact with regulatory RNAs, proteins, etc. It is sometimes but not always true that viral mRNAs are translated more efficiently than cellular mRNAs. I persist in believing that many viruses inhibit cellular protein synthesis inadvertently, and gain little thereby. Understanding the mechanism of host shutoff is nonetheless interesting. It might aid in designing virus vectors, and in our understanding of the conditions that promote persistent virus infections (Ahmed and Fields, 1982).

Acknowledgments

I thank many colleagues who kindly sent reprints and preprints, and especially those who gave me permission to cite their unpublished observations. In several instances the

[19] Recent data from Joram Piatigorsky's laboratory (J. M. Nickerson *et al.*, 1985, and personal communication) suggest that the two forms of δ-crystallin in the embryonic chicken lens are produced from a single mRNA species, by initiating at the first (suboptimal) and second AUG codons. This may be the first example of a cellular mRNA that uses the leaky scanning principle.

soon-to-be published data provide fundamentally new insights and potential solutions to long-standing problems, which augers well for where the field is headed. I am grateful to Aaron Shatkin, Harold Varmus, and Richard Katz for helpful comments on the manuscript, and to W. A. M. for inspiration. Studies in my laboratory are supported by NIH Research Grant GM33915.

REFERENCES

Abraham, G., Rhodes, D., and Banerjee, A. K. (1975). The 5'-terminal structure of the methylated mRNA synthesized *in vitro* by VSV. *Cell* **5,** 51–58.

Abreu, S. L., and Lucas-Lenard, J. (1976). Cellular protein synthesis shutoff by mengovirus: Translation of nonviral and viral mRNAs in extracts from uninfected and infected ascites tumor cells. *J. Virol.* **18,** 182–194.

Ahmad-Zadeh, C., Allet, B., Greenblatt, J., and Weil, R. (1976). Two forms of SV40 T-antigen in abortive and lytic infection. *Proc. Natl. Acad. Sci. U.S.A.* **73,** 1097–1101.

Ahmed, R., and Fields, B. N. (1982). Role of the S4 gene in the establishment of persistent reovirus infection in L cells. *Cell* **28,** 605–612.

Alonso, M. A., and Carrasco, L. (1981). Reversion by hypotonic medium of the shutoff of protein synthesis induced by EMC virus. *J. Virol.* **37,** 535–540.

Alonso, M. A., and Carrasco, L. (1982a). Translation of capped viral mRNAs in poliovirus-infected HeLa cells. *EMBO J.* **1,** 913–917.

Alonso, M. A., and Carrasco, L. (1982b). Protein synthesis in HeLa cells double-infected with EMC virus and poliovirus. *J. Gen. Virol.* **61,** 15'-24.

Alonso, M. A., and Carrasco, L. (1982c). Translation of capped virus mRNA in EMC virus-infected cells. *J. Gen. Virol.* **60,** 315–325.

Anderson, C. W., and Buzash-Pollert, E. (1985). Can ACG serve as an initiation codon for protein synthesis in eukaryotic cells? *Mol. Cell. Biol.* **5,** 3621–3624.

Antczak, J., Chmelo, R., Pickup, D., and Joklik, W. K. (1982). Sequences at both termini of the 10 genes of reovirus serotype 3 (Dearing). *Virology* **121,** 307–319.

Atkins, G. J. (1976). The effect of infection with Sindbis virus and its temperature sensitive mutants on cellular protein and DNA synthesis. *Virology* **71,** 593–597.

Auperin, D., Romanowski, V., Galinski, M., and Bishop, D. H. L. (1984). Sequencing studies of Pichinde arenavirus S RNA indicate a novel coding strategy, an ambisense viral S RNA. *J. Virol.* **52,** 897–904.

Babich, A., Feldman, L., Nevins, J., Darnell, J. E., and Weinberger, C. (1983). Effect of adenovirus on metabolism of specific host mRNAs: Transport control and specific translational discrimination. *Mol. Cell. Biol.* **3,** 1212–1221.

Babiss, L. E., and Ginsberg, H. S. (1984). Adenovirus type 5 early region 1b gene product is required for efficient shutoff of host protein synthesis. *J. Virol.* **50,** 202–212.

Babiss, L. E., Ginsberg, H. S., and Darnell, J. E. (1985). Adenovirus E1B proteins are required for accumulation of late viral mRNA and for effects on cellular mRNA translation and transport. *Mol. Cell. Biol.* **5,** 2552–2558.

Bablanian, R., and Banerjee, A. K. (1986). Polyriboadenylic acid preferentially inhibits *in vitro* translation of cellular compared to vaccinia virus mRNAs. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 1290–1294.

Bablanian, R., Coppola, G., Scribani, S., and Esteban, M. (1981). Inhibition of protein synthesis by vaccinia virus. *Virology* **112,** 13–24.

Ball, L. A., and White, C. N. (1978). Coupled transcription and translation in mammalian and avian cell-free systems. *Virology* **84,** 479–495.

Baltimore, D. (1969). The replication of picornaviruses. *In* "The Biochemistry of Viruses" (H. B. Levy, ed.), pp. 101–176. Dekker, New York.

Bandyopadhyay, P. K., and Temin, H. M. (1984). Expression from an internal AUG codon of herpes simplex thymidine kinase gene inserted in a retrovirus vector. *Mol. Cell. Biol.* **4,** 743–748.

Barkan, A., and Mertz, J. E. (1984). The number of ribosomes on SV40 late 16S mRNA is determined in part by the nucleotide sequence of its leader. *Mol. Cell. Biol.* **4,** 813–816.

Barker, R. F., Jarvis, N. P., Thompson, D., Loesch-Fries, L., and Hall, T. C. (1983). Complete nucleotide sequence of alfalfa mosaic virus RNA 3. *Nucleic Acids Res.* **11,** 2881–2891.

Baroudy, B., Ticehurst, J., Miele, T., Maizel, J., Purcell, R., and Feinstone, S. M. (1985). Sequence analysis of hepatitis A virus cDNA coding for capsid proteins and RNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 2143–2147.

Becerra, S. P., Rose, J. A., Hardy, M., Baroudy, B., and Anderson, C. W. (1985). Direct mapping of adeno-associated virus capsid proteins B and C: A possible ACG initiation codon. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 7919–7923.

Beck, E., Forss, S., Strebel, K., Cattaneo, R., and Feil, G. (1983). Structure of the FMDV translation initiation site and of the structural proteins. *Nucleic Acids Res.* **11,** 7873–7885.

Beier, H., Barciszewska, M., Krupp, G., Mitnacht, R., and Gross, H. J. (1984a). UAG readthrough during TMV RNA translation: Isolation and sequence of two $tRNAs^{Tyr}$ with suppressor activity from tobacco plants. *EMBO J.* **3,** 351–356.

Beier, H., Barciszewska, M., and Sickinger, H-D. (1984b). The molecular basis for differential translation of TMV RNA in tobacco and wheat germ. *EMBO J.* **3,** 1091–1096.

Bellini, W., Englund, G., Rozenblatt, S., Arnheiter, H., and Richardson, C. D. (1985). Measles virus P gene codes for two proteins. *J. Virol.* **53,** 908–919.

Beltz, G. A., and Flint, S. J. (1979). Inhibition of HeLa cell protein synthesis during adenovirus infection. *J. Mol. Biol.* **131,** 353–373.

Bendig, M., Thomas, T., and Folk, W. R. (1980). Regulatory mutants of polyoma virus defective in DNA replication and the synthesis of early proteins. *Cell* **20,** 401–409.

Ben-Hamida, F., Person, A., and Beaud, G. (1983). Solubilization of a protein synthesis inhibitor from vaccinia virions. *J. Virol.* **45,** 452–455.

Bennetzen, J. L., and Hall, B. D. (1982). Codon selection in yeast. *J. Biol. Chem.* **257,** 3026–3031.

Berkhout, B., Kastelein, R., and van Duin, J. (1985). Translational interference at overlapping reading frames in prokaryotic mRNA. *Gene* **37,** 171–179.

Berkner, K., and Sharp, P. A. (1985). Effect of the tripartite leader on synthesis of a nonviral protein in an adenovirus 5 recombinant. *Nucleic Acids Res.* **13,** 841–857.

Bernstein, H. D., Sonenberg, N., and Baltimore, D. (1985). Poliovirus mutant that does not selectively inhibit host cell protein synthesis. *Mol. Cell. Biol.* **5,** 2913–2923.

Bhat, R., and Thimmappaya, B. (1983). Two small RNAs encoded by Epstein–Barr virus can functionally substitute for the virus-associated RNAs in the lytic growth of adenovirus 5. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 4789–4793.

Bhat, R. A., and Thimmappaya, B. (1985). Construction and analysis of additional adenovirus substitution mutants confirm the complementation of VA_I RNA function by two small RNAs encoded by Epstein–Barr virus. *J. Virol.* **56,** 750–756.

Bhat, R., Domer, P., and Thimmappaya, B. (1985). Structural requirements of adenovirus VAI RNA for its translation enhancement function. *Mol. Cell. Biol.* **5,** 187–196.

Bienz, K., Egger, D., Rasser, Y., and Loeffler, H. (1978). Differential inhibition of host cell RNA synthesis in several picornavirus-infected cell lines. *Intervirology* **10,** 209–220.

Bonneau, A.-M., Darveau, A., and Sonenberg, N. (1985). Effect of viral infection on host protein synthesis and mRNA association with the cytoplasmic cytoskeletal structure. *J. Cell Biol.* **100,** 1209–1218.

Boone, R., Parr, R., and Moss, B. (1979). Intermolecular duplexes formed from polyadenylated vaccinia virus RNA. *J. Virol.* **30,** 365–374.

Bos, J., Polder, L., Bernards, R., Schrier, P., van den Elsen, P., van der Eb, A. J., and van Ormondt, H. (1981). The 2.2 kb E1B mRNA of human Ad12 and Ad5 codes for two tumor antigens starting at different AUG triplets. *Cell* **27,** 121–131.

Bossart, W., and Bienz, K. (1981). Regulation of protein synthesis in HEp-2 cells and their cytoplasmic extracts after poliovirus infection. *Virology* **111,** 555–567.

Bouloy, M., Plotch, S., and Krug, R. (1978). Globin mRNAs are primers for the transcription of influenza viral RNA *in vitro*. *Proc. Natl. Aca. Sci. U.S.A.* **75,** 4886–4890.

Bouloy, M., Plotch, S., and Krug, R. (1980). Both the 7-methyl and the 2′-*O*-methyl groups in the cap of mRNA strongly influence its ability to act as primer for influenza virus RNA transcription. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 3952–3956.

Bouloy, M., Vialat, P., Girard, M., and Pardigon, N. (1984). A transcript from the S segment of the Germiston bunyavirus is uncapped and codes for the nucleoprotein and a nonstructural protein. *J. Virol.* **49,** 717–723.

Boursnell, M., Binns, M., and Brown, T. (1985). Sequencing of coronavirus IBV genomic RNA: Three open reading frames in the 5′ "unique" region of mRNA D. *J. Gen. Virol.* **66,** 2253–2258.

Brown, B. A., and Ehrenfeld, E. (1979). Translation of poliovirus RNA *in vitro:* Changes in cleavage pattern and initiation sites by ribosomal salt wash. *Virology* **97,** 396–405.

Brown, B. A., and Ehrenfeld, E. (1980). Initiation factor preparations from poliovirus-infected cells restrict translation in reticulocyte lysates. *Virology* **103,** 327–339.

Brown, G. D., and Moyer, R. W. (1983). The white pock mutants of rabbit poxvirus: *In vitro* translation of early host range mutant mRNA. *Virology* **126,** 381–390.

Brzeski, H., and Kennedy, S. I. T. (1978). Synthesis of alphavirus-specified RNA. *J. Virol.* **25,** 630–640.

Buchman, A. R., Fromm, M., and Berg, P. (1984). Complex regulation of SV40 early-region transcription from different overlapping promoters. *Mol. Cell. Biol.* **4,** 1900–1914.

Budzilowicz, C., Wilczynski, S., and Weiss, S. R. (1985). Three intergenic regions of coronavirus mouse hepatitis virus genome RNA contain a sequence that is homologous to the 3′-end of the viral mRNA leader sequence. *J. Virol.* **53,** 834–840.

Callahan, P., Mizutani, S., and Colonno, R. J. (1985). Molecular cloning and complete sequence determination of RNA genome of human rhinovirus type 14. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 732–736.

Carrasco, L., and Lacal, J. C. (1983). Permeabilization of cells during animal virus infection. *Pharmacol. Ther.* **23,** 109–145.

Carrasco, L., and Smith, A. E. (1976). Sodium ions and the shut-off of host cell protein synthesis by picornaviruses. *Nature (London)* **264,** 807–809.

Cashdollar, L. W., Chmelo, R., Wiener, J., and Joklik, W. K. (1985). Sequences of the S1 genes of the three serotypes of reovirus. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 24–28.

Castiglia, C. L., and Flint, S. J. (1983). Effects of adenovirus infection on rRNA synthesis and maturation in HeLa cells. *Mol. Cell. Biol.* **3,** 662–671.

Castle, E., Nowak, T., Leidner, U., Wengler, G., and Wengler, G. (1985). Sequence analysis of the viral core protein and membrane proteins of the flavivirus West Nile virus. *Virology* **145,** 227–236.

Castle, E., Leidner, U., Nowak, T., Wengler, G., and Wengler, G. (1986). Primary structure of the West Nile flavivirus genome region coding for all nonstructural proteins. *Virology* **149,** 10–26.

Celma, M. L., and Ehrenfeld, E. (1974). Effect of poliovirus double-stranded RNA on viral and host-cell protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **71,** 2440–2444.

Celma, M. L., and Ehrenfeld, E. (1975). Translation of poliovirus RNA *in vitro:* Detection of two different initiation sites. *J. Mol. Biol.* **98,** 761–780.

Centrella, M., and Lucas-Lenard, J. (1982). Regulation of protein synthesis in VSV-infected cells by decreased initiation factor 2 activity. *J. Virol.* **41,** 781–791.

Cepko, C. L., and Sharp, P. A. (1982). Assembly of adenovirus major capsid protein is mediated by a nonvirion protein. *Cell* **31,** 407–415.

Cherney, C. S., and Wilhelm, J. M. (1979). Differential translation in normal and adenovirus type 5-infected cells and cell-free systems. *J. Virol.* **30,** 533–542.

Clarke, B., Sangar, D. V., Burroughs, J., Newton, S., Carroll, A. R., and Rowlands, D. J. (1985). Two initiation sites for foot and mouth disease virus polyprotein in vivo. *J. Gen. Virol.* **66,** 2615–2626.

Collins, P. L., Fuller, F. J., Marcus, P. I., Hightower, L. E., and Ball, L. A. (1982). Synthesis and processing of Sindbis virus nonstructural proteins *in vitro*. *Virology* **118,** 363–379.

Contreras, R., Rogiers, R., Van de Voorde, A., and Fiers, W. (1977). Overlapping of the VP_2-VP_3 gene and the VP_1 gene in the SV40 genome. *Cell* **12,** 529–538.

Cooper, J. A., and Moss, B. (1978). Transcription of vaccinia virus mRNA coupled to translation *in vitro*. *Virology* **88,** 149–165.

Cooper, J. A., and Moss, B. (1979). *In vitro* translation of immediate early, early, and late classes of RNA from vaccinia virus-infected cells. *Virology* **96,** 368–380.

Coppola, G., and Bablanian, R. (1983). Discriminatory inhibition of protein synthesis in cell-free systems by vaccinia virus transcripts. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 75–79.

Craigen, W. J., Cook, R., Tate, W., and Caskey, C. T. (1985). Bacterial peptide chain release factors: Conserved primary structure and possible frameshift regulation of release factor 2. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 3616–3620.

Cross, R. K. (1983). Identification of a unique guanine-7-methyltransferase in Semliki Forest virus infected cell extracts. *Virology* **130,** 452–463.

Daher, K., and Samuel, C. E. (1982). Mechanism of interferon action: Differential effect of interferon on the synthesis of SV40 and reovirus polypeptides in monkey kidney cells. *Virology* **117,** 379–390.

Darlix, J.-L., Zuker, M., and Spahr, P.-F. (1982). Structure–function relationship of Rous sarcoma virus leader RNA. *Nucleic Acids Res.* **10,** 5183–5196.

Das, A., and Yanofsky, C. (1984). A ribosome binding site sequence is necessary for efficient expression of the distal gene of a translationally-coupled gene pair. *Nucleic Acids Res.* **12,** 4757–4768.

Dasgupta, R., Shih, D. S., Saris, C., and Kaesberg, P. (1975). Nucleotide sequence of a viral RNA fragment that binds to eukaryotic ribosomes. *Nature (London)* **256,** 624–628.

Dasgupta, R., Ahlquist, P., and Kaesberg, P. (1980). Sequence of the 3′-untranslated region of brome mosaic virus coat protein messenger RNA. *Virology* **104,** 339–346.

Davies, J. W., and Hull, R. (1982). Genome expression of plant positive-strand RNA viruses. *J. Gen. Virol.* **61,** 1–14.

DeBenedetti, A., and Baglioni, C. (1984). Inhibition of mRNA binding to ribosomes by localized activation of dsRNA-dependent protein kinase. *Nature (London)* **311,** 79–81.

Dethlefsen, L., and Kolakofsky, D. (1983). In vitro synthesis of the nonstructural C protein of Sendai virus. *J. Virol.* **46,** 321–324.

Detjen, B. M., Walden, W. E., and Thach, R. E. (1982). Translational specificity in reovirus-infected mouse fibroblasts. *J. Biol. Chem.* **257,** 9855–9860.

Dijkema, R., Dekker, B., and van Ormondt, H. (1982). Gene organization of the transforming region of adenovirus type 7 DNA. *Gene* **18,** 143–156.

Dixon, L. K., and Hohn, T. (1984). Initiation of translation of the cauliflower mosaic virus genome from a polycistronic mRNA: Evidence from deletion mutagenesis. *EMBO J.* **3,** 2731–2736.

Dixon, L., Jiricny, J., and Hohn, T. (1986). Oligonucleotide directed mutagenesis of cauliflower mosaic virus DNA using a repair-resistant nucleoside analogue identifies an agnogene initiation codon. *Gene,* in press.

Dorner, A. J., Semler, B., Jackson, R., Hanecak, R., Duprey, E., and Wimmer, E. (1984). *In vitro* translation of poliovirus RNA: Utilization of internal initiation sites in reticulocyte lysate. *J. Virol.* **50,** 507–514.

Downey, J. F., Evelegh, C., Branton, P., and Bayley, S. T. (1984). Peptide maps and N-terminal sequences of polypeptides from early region 1A of human adenovirus 5. *J. Virol.* **50,** 30–37.

Dratewka-Kos, E., Kiss, I., Lucas-Lenard, J., Mehta, H., Woodley, C., and Wahba, A. J. (1984). Catalytic utilization of eIF-2 and mRNA binding proteins are limiting in lysates from VSV infected L cells. *Biochemistry* **23,** 6184–6190.

Drillien, R., Spehner, D., and Kirn, A. (1978). Host range restriction of vaccinia virus in CHO cells: Relationship to shutoff of protein synthesis. *J. Virol.* **28,** 843–850.

Duncan, R., and Hershey, J. W. B. (1985a). Regulation of initiation factors during translational repression caused by serum depletion. *J. Biol. Chem.* **260,** 5486–5492.

Duncan, R., and Hershey, J. W. B. (1985b). Cellular levels and covalent modification of the subunits of the cap binding protein complex, eIF-4F. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **44,** 1224.

Duncan, R., Etchison, D., and Hershey, J. W. B. (1983). Protein synthesis factors 4A and 4B are not altered by poliovirus infection of HeLa Cells. *J. Biol. Chem.* **258,** 7236–7239.

Dunn, J. J., and Studier, F. W. (1983). Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J. Mol. Biol.* **166,** 477–535.

Edery, I., Lee, K., and Sonenberg, N. (1984). Functional characterization of eukaryotic mRNA cap binding protein complex: Effects on translation of capped and naturally uncapped RNAs. *Biochemistry* **23,** 2456–2462.

Edwards, S. A., and Fan, H. (1980). Sequence relationship of glycosylated and unglycosylated *gag* polyproteins of Moloney murine leukemia virus. *J. Virol.* **35,** 41–51.

Ehresmann, D. W., and Schaffer, F. L. (1979). Calicivirus intracellular RNA. *Virology* **95,** 251–255.

Enders, G. H., Ganem, D., and Varmus, H. (1985). Mapping the major transcripts of ground squirrel hepatitis virus: The presumptive template for reverse transcriptase is terminally redundant. *Cell* **42,** 297–308.

Ernst, H., and Shatkin, A. J. (1985). Reovirus hemagglutinin mRNA codes for two polypeptides in overlapping reading frames. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 48–52.

Eshita, Y., and Bishop, D. H. L. (1984). The complete sequence of the M RNA of snowshoe hare bunyavirus reveals the presence of internal hydrophobic domains in the viral glycoprotein. *Virology* **137,** 227–240.

Eshita, Y., Ericson, B., Romanowski, V., and Bishop, D. H. L. (1985). Analyses of the mRNA transcription processes of snowshoe hare bunyavirus S and M RNA species. *J. Virol.* **55,** 681–689.

Etchison, D., and Fout, S. (1985). Human rhinovirus 14 infection of HeLa cells results in the proteolytic cleavage of the p220 cap binding subunit. *J. Virol.* **54,** 634–638.

Etchison, D., Milburn, S., Edery, I., Sonenberg, N., and Hershey, J. W. B. (1982). Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with

proteolysis of a 220,000 dalton polypeptide associated with eIF3 and a cap binding protein complex. *J. Biol. Chem.* **257,** 14806–14810.

Etchison, D., Hansen, J., Ehrenfeld, E., Edery I., Sonenberg, N., Milburn, S., and Hershey, J. W. B. (1984). Demonstration in vitro that eIF3 is active but that a cap-binding protein complex is inactive in poliovirus-infected HeLa cells. *J. Virol.* **51,** 832–837.

Fenwick, M. L., and McMenamin, M. M. (1984). Early virion-associated suppression of cellular protein synthesis by herpes simplex virus is accompanied by inactivation of mRNA. *J. Gen. Virol.* **65,** 1225–1228.

Fenwick, M. L., and Walker, M. J. (1978). Suppression of the synthesis of cellular macromolecules by herpes simplex virus. *J. Gen. Virol.* **41,** 37–51.

Fernandez-Munoz, R., and Darnell, J. E. (1976). Structural difference between the 5′ termini of viral and cellular mRNA in poliovirus-infected cells: Possible basis for the inhibition of host protein synthesis. *J. Virol.* **18,** 719–726.

Flint, S. J., Plumb, M. A., Yang, U.-C., Stein, G. S., and Stein, J. L. (1984). Effect of adenovirus infection on expression of human histone genes. *Mol. Cell. Biol.* **4,** 1363–1371.

Forss, S., Strebel, K., Beck, E., and Schaller, H. (1984). Nucleotide sequence and genome organization of foot and mouth disease virus. *Nucleic Acids Res.* **12,** 6587–6601.

Fraenkel-Conrat, H., and Wagner, R. R., eds. (1984). "Comprehensive Virology," Vol 19. Plenum, New York.

Francoeur, A. M., and Stanners, C. P. (1978). Evidence against the role of K^+ in the shutoff of protein synthesis by VSV. *J. Gen. Virol.* **39,** 551–554.

Friesen, P. D., and Miller, L. K. (1985). Temporal regulation of baculovirus RNA: Overlapping early and late transcripts. *J. Virol.* **54,** 392–400.

Friesen, P. D., and Rueckert, R. R. (1984). Early and late functions in a bipartite RNA virus: Evidence for translational control by competition between viral mRNAs. *J. Virol.* **49,** 116–124.

Fuller, F., Bhown, A., and Bishop, D. H. L. (1983). Bunyavirus nucleoprotein, N, and a nonstructural protein, NS_S, are coded by overlapping reading frames in the S RNA. *J. Gen. Virol.* **64,** 1705–1714.

Furuichi, Y., Morgan, M., Muthukrishnan, S., and Shatkin, A. J. (1975). Reovirus messenger RNA contains a methylated, blocked 5′-terminal structure: $m^7G(5')ppp(5')G^mpCp$-. *Proc. Natl. Acad. Sci. U.S.A.* **72,** 362–366.

Furuichi, Y., Muthukrishnan, S., Tomasz, J., and Shatkin, A. J. (1976). Mechanism of formation of reovirus mRNA 5′-terminal blocked and methylated sequence, m^7GpppG^mpC. *J. Biol. Chem.* **251,** 5043–5053.

Furuichi, Y., LaFiandra, A., and Shatkin, A. J. (1977). 5′-Terminal structure and mRNA stability. *Nature (London)* **266,** 235–239.

Furukawa, T., Jean, J.-H., and Plotkin, S. A. (1978). Enhanced poliovirus replication in cytomegalovirus-infected human fibroblasts. *Virology* **85,** 622–625.

Garry, R. F., and Waite, M. R. F. (1979). Na^+ and K^+ concentrations and the regulation of the interferon system in chick cells. *Virology* **96,** 121–128.

Garry, R. F., Bishop, J. M., Parker, S., Westbrook, K., Lewis, G., and Waite, M. R. F. (1979). Na^+ and K^+ concentrations and the regulation of protein synthesis in Sindbis virus-infected chick cells. *Virology* **96,** 108–120.

Gehrke, L., Auron, P. E., Quigley, G. J., Rich, A., and Sonenberg, N. (1983). 5′-Conformation of capped alfalfa mosaic virus RNA 4 may reflect its independence of cap structure or of cap-binding protein for efficient translation. *Biochemistry* **22,** 5157–5164.

Ghosh, P. K., and Lebowitz, P. (1981). SV40 early mRNAs contain multiple 5′-termini

upstream and downstream from a Hogness–Goldberg sequence; a shift in 5′ termini during the lytic cycle is mediated by large T antigen. *J. Virol.* **40,** 224–240.

Ghosh, P. K., Reddy, V. B., Swinscoe, J., Lebowitz, P., and Weissman, S. M. (1978). Heterogeneity and 5′-terminal structures of the late RNAs of SV40. *J. Mol. Biol.* **126,** 813–846.

Gillies, S., and Stollar, V. (1982). Protein synthesis in lysates of *Aedes albopictus* cells infected with VSV. *Mol. Cell. Biol.* **2,** 1174–1186.

Giorgi, C., Blumberg, B. M., and Kolakofsky, D. (1983). Sendai virus contains overlapping genes expressed from a single mRNA. *Cell* **35,** 829–836.

Godefroy-Colburn, T., Thivent, C., and Pinck, L. (1985a). Translational discrimination between the four RNAs of alfalfa mosaic virus. *Eur. J. Biochem.* **147,** 541–548.

Godefroy-Colburn, T., Ravelonandro. M., and Pinck, L. (1985b). Cap accessibility correlates with the initiation efficiency of AMV RNAs. *Eur. J. Biochem.* **147,** 549–552.

Goelet, P., Lomonossoff, G., Butler, P., Akam, M., Gait, M., and Karn, J. (1982). Nucleotide sequence of tobacco mosaic virus RNA. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5818–5822.

Golini, F., Thach, S. S., Birge, C. H., Safer, B., Merrick, W. C., and Thach, R. E. (1976). Competition between cellular and viral mRNAs *in vitro* is regulated by a messenger discriminatory initiation factor. *Proc. Natl. Acad. Sci. U.S.A.* **73,** 3040–3044.

Gray, M. A., Micklem, K. J., and Pasternak, C. A. (1983). Protein synthesis in cells infected with Semliki Forest virus is not controlled by intracellular cation changes. *Eur. J. Biochem.* **135,** 299–302.

Grifo, J. A., Tahara, S. M., Morgan, M. A., Shatkin, A. J., and Merrick, W. C. (1983). New initiation factor activity required for globin mRNA translation. *J. Biol. Chem.* **258,** 5804–5810.

Grinnell, B. W., and Wagner, R. R. (1985). Inhibition of DNA-dependent transcription by the leader RNA of VSV. *Mol. Cell. Biol.* **5,** 2502–2513.

Gruss, P., Ellis, R., Shih, T., Konig, M., Scolnick, E. M., and Khoury, G. (1981). SV40 recombinant molecules express the gene encoding p21 transforming protein of Harvey murine sarcoma virus. *Nature (London)* **293,** 486–488.

Guarino, L. A., Ghosh, A., Dasmahapatra, B., Dasgupta, R., and Kaesberg, P. (1984). Sequence of the black beetle virus subgenomic RNA and its location in the viral genome. *Virology* **139,** 199–203.

Guilley, H., Carrington, J. C., Balazs, E., Jonard, G., Richards, K., and Morris, T. J. (1985). Nucleotide sequence and genome organization of carnation mottle virus RNA. *Nucleic Acids Res.* **13,** 6663–6677.

Haarr, L., Marsden, H., Preston, C. M., Smiley, J. R., Summers, W. C., and Summers, W. P. (1985). Utilization of internal AUG codons for initiation of protein synthesis directed by mRNAs from normal and mutant genes encoding herpes simplex virus-specified thymidine kinase. *J. Virol.* **56,** 512–519.

Hackett, P. B., Petersen, R. B., Hensel, C., Albericio, F., Gunderson, S., Palmenberg, A., and Barany, G. (1986). Synthesis *in vitro* of a seven amino acid peptide encoded in the leader RNA of Rous sarcoma virus. *J. Mol. Biol.*, in press.

Hackstadt, T., and Mallavia, L. P. (1982). Sodium and potassium transport in herpes simplex virus-infected cells. *J. Gen. Virol.* **60,** 199–207.

Halbert, D. N., Cutt, J. R., and Shenk, T. (1985). Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. *J. Virol.* **56,** 250–257.

Halpern, M. E., and Smiley, J. R. (1984). Effects of deletions on expression of the HSV thymidine kinase gene from the intact viral genome: The amino terminus is dispensable for catalytic activity. *J. Virol.* **50,** 733–738.

Hay, A. J., Abraham, G., Skehel, J., Smith, J. C., and Fellner, P. (1977). Influenza virus mRNAs are incomplete transcripts of the genome RNAs. *Nucleic Acids Res.* **4,** 4197–4209.

Hay, N., and Aloni, Y. (1985). Attenuation of late SV40 mRNA synthesis is enhanced by the agnoprotein and is temporally regulated in isolated nuclear systems. *Mol. Cell. Biol.* **5,** 1327–1334.

Heermann, K. H., Goldmann, U., Schwartz, W., Seyffarth, T., Baumgarten, H., and Gerlich, W. H. (1984). Large surface proteins of hepatitis V virus containing the pre-S sequence. *J. Virol.* **52,** 396–402.

Helentjaris, T., and Ehrenfeld, E. (1977). Inhibition of host cell protein synthesis by UV-inactivated poliovirus. *J. Virol.* **21,** 259–267.

Helentjaris, T., and Ehrenfeld, E. (1978). Control of protein synthesis in extracts from poliovirus-infected cells. *J. Virol.* **26,** 510–521.

Hewlett, M. J., Axelrod, J. H., Antinoro, N., and Feld, R. (1982). Isolation and preliminary characterization of temperature-sensitive mutants of poliovirus type 1. *J. Virol.* **41,** 1089–1094.

Hill, T. M., Sadler, J. R., and Betz, J. L. (1985). Virion component of HSV type 1 KOS interferes with early shutoff of host protein synthesis induced by HSV type 2. *J. Virol.* **56,** 312–316.

Hiremath, L. S., Webb, N. R., and Rhoads, R. E. (1985). Immunological detection of the mRNA cap-binding protein. *J. Biol. Chem.* **260,** 7843–7849.

Hoess, R. H., Foeller, C., Bidwell, K., and Landy, A. (1980). Site-specific recombination of bacteriophage λ: DNA sequence of regulatory regions and overlapping structural genes for Int and Xis. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 2482–2486.

Horikami, S. M., De Ferra, F., and Moyer, S. A. (1984). Characterization of the infections of permissive cells by host range mutants of VSV defective in RNA methylation. *Virology* **138,** 1–15.

Hruby, D. E., and Ball, L. A. (1981). Control of expression of the vaccinia virus thymidine kinase gene. *J. Virol.* **40,** 456–464.

Hruby, D. E., and Ball, L. A. (1982). Mapping and identification of the vaccinia virus thymidine kinase gene. *J. Virol.* **43,** 403–409.

Hughes, S., Mellstrom, K., Kosik, E., Tamanoi, F., and Brugge, J. (1984). Mutation of a termination codon affects src initiation. *Mol. Cell. Biol.* **4,** 1738–1746.

Huisman, M. J., Sarachu, A. N., Alblas, F., and Bol, J. F. (1985). Alfalfa mosaic virus temperature sensitive mutants. *Virology* **141,** 23–29.

Ikegami, S., Takahashi, N., Itoh, N., Higaki, T., and Sasaki, S. (1985). Transcription-coupled translation of silkworm cytoplasmic polyhedrosis virus genomic RNA in *Xenopus* oocytes. *Int. Congr. Biochem., 13th, Amsterdam* Abstr. MO-111.

Ikemura, T. (1981). Correlation between the abundance of *E. coli* transfer RNAs and the occurrence of the respective codons in its protein genes. *J. Mol. Biol.* **146,** 1–21.

Ikemura, T. (1982). Correlation between the abundance of yeast transfer RNAs and the occurrence of the respective codons in protein genes. *J. Mol. Biol.* **158,** 573–597.

Inglis, S. C. (1982). Inhibition of host protein synthesis and degradation of cellular mRNAs during infection by influenza and HSV. *Mol. Cell. Biol.* **2,** 1644–1648.

Izant, J. G., and Weintraub, H. (1985). Constitutive and conditional suppression of exogenous and endogenous genes by anti-sense RNA. *Science* **229,** 345–352.

Jacks, T., and Varmus, H. E. (1985). Expression of the Rous sarcoma virus *pol* gene by ribosomal frameshifting. *Science* **230,** 1237–1242.

Jacobs, B. L., and Samuel, C. E. (1985). Biosynthesis of reovirus-specified polypeptides: The reovirus s1 mRNA encodes two primary translation products. *Virology* **143,** 63–74.

Jacobs, B. L., Atwater, J. A., Munemitsu, S. M., and Samuel, C. E. (1985). Biosynthesis of reovirus-specified polypeptides. *Virology* **147,** 9–18.

Jacobson, M. F., and Baltimore, D. (1968). Polypeptide cleavages in the formation of poliovirus proteins. *Proc. Natl. Acad. Sci. U.S.A.* **61,** 77–84.

Janik, J. E., Huston, M. M., Cho, K., and Rose, J. A. (1982). Requirement for adenovirus DNA-binding protein and VA-I RNA for production of adeno-associated virus polypeptides. *J. Cell Biochem. (Suppl.)* **6,** 209.

Janik, J. E., Huston, M. M., and Rose, J. A. (1984). Adeno-associated virus proteins: Origin of the capsid components. *J. Virol.* **52,** 591–597.

Jay, G., Nomura, S., Anderson, C. W., and Khoury, G. (1981). Identification of the SV40 agnogene product: A DNA binding protein. *Nature (London)* **291,** 346–349.

Jaye, M. C., Godchaux, W., and Lucas-Lenard, J. (1982). Further studies on the inhibition of cellular protein synthesis by VSV. *Virology* **116,** 148–162.

Jen, G., and Thach, R. E. (1982). Inhibition of host translation in EMC virus-infected L cells: A novel mechanism. *J. Virol.* **43,** 250–261.

Jen, G., Birge, C. H., and Thach, R. E. (1978). Comparison of initiation rates of EMC virus and host protein synthesis in infected cells. *J. Virol.* **27,** 640–647.

Jen, G., Detjen, B. M., and Thach, R. E. (1980). Shutoff of HeLa cell protein synthesis by EMC virus and poliovirus: A comparative study. *J. Virol.* **35,** 150–156.

Jense, H., Knauert, F., and Ehrenfeld, E. (1978). Two initiation sites for translation of poliovirus RNA *in vitro:* Comparison of LSc and Mahoney strains. *J. Virol.* **28,** 387–394.

Johnson, D. C., and Spear, P. G. (1984). Evidence for translational regulation of HSV type 1 gD expression. *J. Virol.* **51,** 389–394.

Jones, E. V., Whitaker-Dowling, P., and Youngner, J. S. (1982). Restriction of VSV in a nonpermissive rabbit cell line is at the level of protein synthesis. *Virology* **121,** 20–31.

Kääriäinen, L., and Ranki, M. (1984). Inhibition of cell functions by RNA-virus infections. *Annu. Rev. Microbiol.* **38,** 91–109.

Kastelein, R. A., Remaut, E., Fiers, W., and van Duin, J. (1982). Lysis gene expression of RNA phage MS2 depends on a frameshift during translation of the overlapping coat protein gene. *Nature (London)* **295,** 35–41.

Katinka, M., and Yaniv, M. (1982). Deletions of N-terminal sequences of polyoma virus T-antigens reduce but do not abolish transformation of rat fibroblasts. *Mol. Cell. Biol.* **2,** 1238–1246.

Katz, R. A., Cullen, B. R., Malavarca, R., and Skalka, A. M. (1986). Role of the avian retrovirus mRNA leader in expression: Evidence for novel translation control. *Mol. Cell. Biol.* **6,** 372–379.

Katze, M. G., and Krug, R. M. (1984). Metabolism and expression of RNA polymerase II transcripts in influenza virus-infected cells. *Mol. Cell. Biol.* **4,** 2198–2206.

Katze, M. G., Chen, Y-T., and Krug, R. M. (1984). Nuclear–cytoplasmic transport and VAI RNA-independent translation of influenza viral mRNAs in late adenovirus-infected cells. *Cell* **37,** 483–490.

Katze, M. G., Detjen, B. M., Safer, B., and Krug, R. M. (1986). Translational control by influenza virus: Suppression of the kinase that phosphorylates the alpha subunit of initiation factor eIF-2 and selective translation of influenza viral mRNAs. *Mol. Cell. Biol.* **6,** in press.

Kaufman, R. J. (1985). Identification of the components necessary for adenovirus translational control and their utilization in cDNA expression vectors. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 689–693.

Kiberstis, P. A., Loesch-Fries, L. S., and Hall, T. C. (1981). Viral protein synthesis in

barley protoplasts inoculated with native and fractionated brome mosaic virus RNA. *Virology* **112,** 804–808.

Kitamura, N., Semler, B., Rothberg, P., Larsen, G., Adler, C., Dorner, A., Emini, E., Hanecak, R., Lee, J., van der Werf, S., Anderson, C. W., and Wimmer, E. (1981). Primary structure, gene organization and polypeptide expression of poliovirus RNA. *Nature (London)* **291,** 547–553.

Knight, E., Anton, E. D., Fahey, D., Friedland, B., and Jonak, G. J. (1985). Interferon regulates c-*myc* gene expression in Daudi cells at the post-transcriptional level. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 1151–1154.

Knowland, J. (1974). Protein synthesis directed by the RNA from a plant virus in a normal animal cell. *Genetics* **78,** 383–394.

Kozak, M. (1978). How do eucaryotic ribosomes select initiation regions in messenger RNA? *Cell* **15,** 1109–1123.

Kozak, M. (1979a). Inability of circular mRNA to attach to eukaryotic ribosomes. *Nature (London)* **280,** 82–85.

Kozak, M. (1979b). Migration of 40S ribosomal subunits on mRNA when initiation is perturbed by lowering magnesium or adding drugs. *J. Biol. Chem.* **254,** 4731–4738.

Kozak, M. (1980). Evaluation of the "scanning model" for initiation of protein synthesis in eucaryotes. *Cell* **22,** 7–8.

Kozak, M. (1981a). Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. *Nucleic Acids Res.* **9,** 5233–5252.

Kozak, M. (1981b). Mechanism of mRNA recognition by eukaryotic ribosomes during intiation of protein synthesis. *Curr. Top. Microbiol. Immunol.* **93,** 81–123.

Kozak, M. (1982). Analysis of ribosome binding sites from the s1 message of reovirus: Initiation at the first and second AUG codons. *J. Mol. Biol.* **156,** 807–820.

Kozak, M. (1983a). Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. *Microbiol. Rev.* **47,** 1–45.

Kozak, M. (1983b). Translation of insulin-related polypeptides from mRNAs with tandemly reiterated copies of the ribosome binding site. *Cell* **34,** 971–978.

Kozak, M. (1984a). Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12,** 857–872.

Kozak, M. (1984b). Selection of initiation sites by eucaryotic ribosomes: Effect of inserting AUG triplets upstream from the coding sequence for preproinsulin. *Nucleic Acids Res.* **12,** 3873–3893.

Kozak, M. (1986a). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eucaryotic ribosomes. *Cell* **44,** 283–292.

Kozak, M. (1986b). Influences of mRNA secondary structure on initiation by eucaryotic ribosomes. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

Kozak, M., and Shatkin, A. J. (1978a). Migration of 40S ribosomal subunits on mRNA in the presence of edeine. *J. Biol. Chem.* **253,** 6568–6577.

Kozak, M., and Shatkin, A. J. (1978b). Identification of features in 5′-terminal fragments from reovirus mRNA which are important for ribosome binding. *Cell* **13,** 201–212.

Krug, R. M. (1981). Priming of influenza viral RNA transcription by capped heterologous RNAs. *Curr. Top. Microbiol. Immunol.* **93,** 125–149.

Lacal, J. C., and Carrasco, L. (1982). Relationship between membrane integrity and the inhibition of host translation in virus-infected mammalian cells. *Eur. J. Biochem.* **127,** 359–366.

Lachmi, B., and Kääriäinen, L. (1977). Control of protein synthesis in Semliki Forest virus-infected cells. *J. Virol.* **22,** 142–149.

Lai, M., Baric, R., Brayton, P., and Stohlman, S. A. (1984). Characterization of leader

RNA sequences on the virion and mRNAs of mouse hepatitis virus, a cytoplasmic RNA virus. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 3626–3630.

Laprevotte, I., Hampe, A., Sherr, C. J., and Galibert, F. (1984). Nucleotide sequence of the *gag* gene and *gag–pol* junction of feline leukemia virus. *J. Virol.* **50,** 884–894.

Laub, O., Rall, L. B., Truett, M., Shaul, Y., Standring, D., Valenzuela, P., and Rutter, W. J. (1983). Synthesis of hepatitis B surface antigen in mammalian cells: Expression of the entire gene and the coding region. *J. Virol.* **48,** 271–280.

Lawrence, C. B., and Jackson, K. J. (1982). Translation of adenovirus serotype 2 late mRNAs. *J. Mol. Biol.* **162,** 317–334.

Lazarowitz, S. G., Compans, R. W., and Choppin, P. W. (1971). Influenza virus structural and nonstructural proteins in infected cells and their plasma membranes. *Virology* **46,** 830–843.

Lee, K. A. W., and Sonenberg, N. (1982). Inactivation of cap-binding proteins accompanies the shut-off of host protein synthesis by poliovirus. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 3447–3451.

Lee, K. A. W., Edery, I., and Sonenberg, N. (1985a). Isolation and structural characterization of cap-binding proteins from poliovirus-infected HeLa cells. *J. Virol.* **54,** 515–524.

Lee, K. A. W., Edery, I., Hanecak, R., Wimmer, E., and Sonenberg, N. (1985b). Poliovirus protease 3C (P3-7c) does not cleave p220 of the eucaryotic mRNA cap-binding protein complex. *J. Virol.* **55,** 489–493.

Lemieux, R., and Beaud, G. (1982). Expression of vaccinia virus early mRNAs in Ehrlich ascites tumor cells. Part of the polysomes at an early stage of virus infection are not bound to the cytoskeleton. *Eur. J. Biochem.* **129,** 273–279.

Lemieux, R., Vassef, A., Ben-Hamida, F., and Beaud, G. (1982). Translation of cellular and viral early mRNA in cell-free systems from uninfected and (vaccinia) virus-infected cells at the early stage. *Eur. J. Biochem.* **129,** 265–271.

Lemieux, R., Zarbl, H., and Millward, S. (1984). mRNA discrimination in extracts from uninfected and reovirus-infected L-cells. *J. Virol.* **51,** 215–222.

LeMoullec, J. M., Akusjarvi, G., Stalhandske, P., Pettersson, U., Chambraud, B., Gilardi, P., Nasri, M., and Perricaudet, M. (1983). Polyadenylic acid addition sites in the adenovirus type 2 major late transcription unit. *J. Virol.* **48,** 127–134.

Lengyel, P. (1982). Biochemistry of interferons and their actions. *Annu. Rev. Biochem.* **51,** 251–282.

Lenk, R., and Penman, S. (1979). The cytoskeletal framework and poliovirus metabolism. *Cell* **16,** 289–301.

Linemeyer, D. L., Menke, J. G., Martin-Gallardo, A., Hughes, J., Young, A., and Mitra, S. (1985). Molecular cloning and partial sequencing of hepatitis A viral cDNA. *J. Virol.* **54,** 247–255.

Liu, C.-C., Simonsen, C. C., and Levinson, A. D. (1984). Initiation of translation at internal AUG codons in mammalian cells. *Nature (London)* **309,** 82–85.

Lizardi, P. M., Mahdavi, V., Shields, D., and Candelas, G. (1979). Discontinuous translation of silk fibroin in a reticulocyte cell-free system and in intact silk gland cells. *Proc. Natl. Acad. Sci. U.S.A.* **76,** 6211–6215.

Lloyd, R. E., Etchison, D., and Ehrenfeld, E. (1985). Poliovirus protease does not mediate cleavage of the 220,000-Da component of the cap binding protein complex. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 2723–2727.

Lodish, H. F., and Porter, M. (1980). Translational control of protein synthesis after infection by VSV. *J. Virol.* **36,** 719–733.

Lodish, H. F., and Porter, M. (1981). VSV mRNA and inhibition of translation of cellular mRNA—is there a P function in VSV? *J. Virol.* **38,** 504–517.

Logan, J., and Shenk, T. (1984). Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 3655–3659.

Lomedico, P. T., and McAndrew, S. J. (1982). Eukaryotic ribosomes can recognize preproinsulin initiation codons irrespective of their position relative to the 5′-end of mRNA. *Nature (London)* **299,** 221–226.

Lopez, S., Bell, J. R., Strauss, E. G., and Strauss, J. H. (1985). The nonstructural proteins of Sindbis virus as studied with an antibody specific for the C terminus of the nonstructural readthrough polyprotein. *Virology* **141,** 235–247.

McAllister, P. E., and Wagner, R. R. (1976). Differential inhibition of host protein synthesis in L cells infected with RNA$^-$ temperature-sensitive mutants of VSV. *J. Virol.* **18,** 550–558.

Mahr, A., and Roberts, B. E. (1984). Arrangment of late RNAs transcribed from a 7.1-kb EcoRI vaccinia virus DNA fragment. *J. Virol.* **49,** 510–520.

Mann, R., Mulligan, R. C., and Baltimore, D. (1983). Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. *Cell* **33,** 153–159.

Mardon, G., and Varmus, H. E. (1983). Frameshift and intragenic suppressor mutations in a Rous sarcoma provirus suggest *src* encodes two proteins. *Cell* **32,** 871–879.

Margolskee, R. F., and Nathans, D. (1983). Suppression of a VP1 mutant of SV40 by missense mutations in serine codons of the viral agnogene. *J. Virol.* **48,** 405–409.

Mathews, M. B., and Grodzicker, T. (1981). Virus-associated RNAs of naturally occurring strains and variants of group C adenoviruses. *J. Virol.* **38,** 849–862.

Meshi, T., Kyama, R., Ohno, T., and Okada, Y. (1983). Nucleotide sequence of the coat protein cistron and the 3′-noncoding region of cucumber green mottle mosaic virus RNA. *Virology* **127,** 54–64.

Minor, P. D., Hart, J. G., and Dimmock, N. J. (1979). Influence of the host cell on proteins synthesized by different strains of influenza virus. *Virology* **97,** 482–487.

Misra, R., and Reeves, P. (1985). Intermediates in the synthesis of To1C protein include an incomplete peptide stalled at a rare Arg codon. *Eur. J. Biochem.* **152,** 151–155.

Moldave, K. (1985). Eukaryotic protein synthesis. *Ann. Rev. Biochem.* **54,** 1109–1149.

Monckton, R. P., and Westaway, E. G. (1982). Restricted translation of the genome of the flavivirus Kunjin *in vitro*. *J. Gen. Virol.* **63,** 227–232.

Monstein, H-J., and Philipson, L. (1981). The conformation of adenovirus VAI-RNA in solution. *Nucleic Acids Res.* **9,** 4239–4250.

Morrow, C. D., Gibbons, G. F., and Dasgupta, A. (1985). The host protein required for *in vitro* replication of poliovirus is a protein kinase that phosphorylates eIF2. *Cell* **40,** 913–921.

Mosenkis, J., Daniels-McQueen, S., Janovec, S., Duncan, R., Hershey, J. W. B., Grifo, J., Merrick, W. C., and Thach, R. E. (1985). Shutoff of host translation by EMC virus infection does not involve cleavage of the eIF4F polypeptide that accompanies poliovirus infection. *J. Virol.* **54,** 643–645.

Moss, B. (1968). Inhibition of HeLa cell protein synthesis by the vaccinia virion. *J. Virol.* **2,** 1028–1037.

Moss, B., and Filler, R. (1970). Irreversible effects of cycloheximide during the early period of vaccinia virus replication. *J. Virol.* **5,** 99–108.

Moss, B., Gershowitz, A., Wei, C.-M., and Boone, R. (1976). Formation of the guanylylated and methylated 5′-terminus of vaccinia virus mRNA. *Virology* **72,** 341–351.

Munemitsu, S. M., and Samuel, C. E. (1984). Biosynthesis of reovirus-specified polypeptides. Multiplication rate but not yield of reovirus serotypes 1 and 3 correlates with the level of virus-mediated inhibition of cellular protein synthesis. *Virology* **136,** 133–143.

Muñoz, A., Alonso, M. A., and Carrasco, L. (1985a). The regulation of translation in reovirus-infected cells. *J. Gen. Virol.* **66,** 2161–2170.

Muñoz, A., Castrillo, J. L., and Carrasco, L. (1985b). Modification of membrane permeability during Semliki Forest virus infection. *Virology* **146,** 203–212.

Murphy, E. C., Kopchick, J. J., Watson, K. F., and Arlinghaus, R. B. (1978). Cell-free synthesis of a precursor polyprotein containing both *gag* and *pol* gene products by Rauscher murine leukemia virus 35S RNA. *Cell* **13,** 359–369.

Nair, C. N., Stowers, J. W., and Singfield, B. (1979). Guanidine-sensitive Na^+ accumulation by poliovirus-infected HeLa cells. *J. Virol.* **31,** 184–189.

Nevins, J. R. (1982). Adenovirus gene expression: Control at multiple steps of mRNA biogenesis. *Cell* **28,** 1–2.

Nickerson, J. M., Wawrousek, E., Hawkins, J., Wakil, A., Wistow, G., Thomas, G., Norman, B. L., and Piatigorsky, J. (1985). The complete sequence of the chicken δ1 crystallin gene and its 5′ flanking region. *J. Biol. Chem.* **260,** 9100–9105.

Nishioka, Y., and Silverstein, S. (1978a). Alterations in the protein synthetic apparatus of Friend erythroleukemia cells infected with VSV or herpes simplex virus. *J. Virol.* **25,** 422–426.

Nishioka, Y., and Silverstein, S. (1978b). Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with HSV type 1. *J. Virol.* **27,** 619–627.

Nishizawa, M., Koyama, T., and Kawai, S. (1985). Unusual features of the leader sequence of Rous sarcoma virus packaging mutant TK15. *J. Virol.* **55,** 881–885.

Nomoto, A., Lee, Y. F., and Wimmer, E. (1976). The 5′-end of poliovirus mRNA is not capped with $m^7G(5')ppp(5')Np$. *Proc. Natl. Acad. Sci. U.S.A.* **73,** 375–380.

Nomoto, A., Omata, T., Toyoda, H., Kuge, S., Horie, H., Kataoka, Y., Genba, Y., Nakano, Y., and Imura, N. (1982). Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5793–5797.

Nonoyama, M., Millward, S., and Graham, A. F. (1974). Control of transcription of the reovirus genome. *Nucleic Acids Res.* **1,** 373–385.

Norrie, D. H., Wolstenholme, J., Howcroft, H., and Stephen, J. (1982). Vaccinia virus-induced changes in $[Na^+]$ and $[K^+]$ in HeLa cells. *J. Gen. Virol.* **62,** 127–136.

Nuss, D. L., Oppermann, H., and Koch, G. (1975). Selective blockage of initiation of host protein synthesis in RNA-virus-infected cells. *Proc. Natl. Acad. Sci. U.S.A.* **72,** 1258–1262.

O'Malley, R. P., Mariano, T. M., Siekierka, J., and Mathews, M. B. (1986). A mechanism for the control of protein synthesis by adenovirus VA RNA_I. *Cell* **44,** 391–400.

Oppermann, H., and Koch, G. (1976). On the regulation of protein synthesis in vaccinia virus infected cells. *J. Gen. Virol.* **32,** 261–273.

Oppermann, H., Bishop, J. M., Varmus, H. E., and Levintow, L. (1977). A joint product of the genes *gag* and *pol* of avian sarcoma virus: A possible precursor of reverse transcriptase. *Cell* **12,** 993–1005.

Oren, M., Maltzman, W., and Levine, A. J. (1981). Post-translational regulation of the 54K cellular tumor antigen in normal and transformed cells. *Mol. Cell. Biol.* **1,** 101–110.

Otto, M. J., and Lucas-Lenard, J. (1980). The influence of the host cell on the inhibition of virus protein synthesis in cells doubly infected with VSV and mengovirus. *J. Gen. Virol.* **50,** 293–307.

Ou, J.-H., Rice, C. M., Dalgarno, L., Strauss, E. G., and Strauss, J. H. (1982). Sequence studies of several alphavirus genomic RNAs in the region containing the start of the subgenomic RNA. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5235–5239.

Pacha, R. F., and Condit, R. C. (1985). Characterization of a *ts* mutant of vaccinia virus

reveals a novel function that prevents virus-induced breakdown of RNA. *J. Virol.* **56,** 395–403.

Pasek, M., Goto, T., Gilbert, W., Zink, B., Schaller, H., MacKay, P., Leadbetter, G., and Murray, K. (1979). Hepatitis B virus genes and their expression in *E. coli. Nature (London)* **282,** 575–579.

Patterson, J. L., and Kolakofsky, D. (1984). Characterization of LaCrosse virus small-genome transcripts. *J. Virol.* **49,** 680–685.

Patterson, J. L., Cabradilla, C., Holloway, B. P., Obijeski, J. F., and Kolakofsky, D. (1983). Multiple leader RNAs and mRNAs are transcribed from the LaCrosse virus small genome segment. *Cell* **33,** 791–799.

Pattnaik, A. K., and Abraham, G. (1983). Identification of four complementary RNA species in Akabane virus-infected cells. *J. Virol.* **47,** 452–462.

Payne, C. C., and Mertens, P. P. C. (1983). *In* "The Reoviridae" (W. K. Joklik, ed.), pp. 425–504. Plenum, New York.

Payvar, F., and Schimke, R. T. (1979). Methylmercury hydroxide enhancement of translation and transcription of ovalbumin and conalbumin mRNAs. *J. Biol. Chem.* **254,** 7636–7642.

Pedersen, S. (1984). *E. coli* ribosomes translate *in vivo* with variable rate. *EMBO J.* **3,** 2895–2898.

Pelham, H. (1978). Leaky UAG termination codon in tobacco mosaic virus RNA. *Nature (London)* **272,** 469–471.

Pelham, H. R. B., Sykes, J. M. M., and Hunt, T. (1978). Characteristics of a coupled cell-free transcription and translation system directed by vaccinia cores. *Eur. J. Biochem.* **82,** 199–209.

Pelletier, J., and Sonenberg, N. (1985). Insertion mutagenesis to increase secondary structure within the 5′-noncoding region of a eukaryotic mRNA reduces translational efficiency. *Cell* **40,** 515–526.

Pensiero, M. N., and Lucas-Lenard, J. M. (1985). Evidence for the presence of an inhibitor on ribosomes in mouse L cells infected with mengovirus. *J. Virol.* **56,** 161–171.

Perdue, M. L., Borchelt, D., and Resnick, R. J. (1982). Participation of 5′-terminal leader sequences in *in vitro* translation of Rous sarcoma virus RNA. *J. Biol. Chem.* **257,** 6551–6555.

Pereira, L., Wolff, M. H., Fenwick, M., and Roizman, B. (1977). Regulation of herpesvirus macromolecular synthesis. Properties of α polypeptides made in HSV-1 and HSV-2 infected cells. *Virology* **77,** 733–749.

Perlman, S., Hirsch, M., and Penman, S. (1972). Utilization of messenger in adenovirus-2-infected cells at normal and elevated temperatures. *Nature (London) New Biol.* **238,** 143–144.

Persing, D. H., Varmus, H. E., and Ganem, D. (1985). A frameshift mutation in the pre-S region of the human hepatitis B virus genome allows production of surface antigen particles but eliminates binding to polymerized albumin. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 3440–3444.

Petersen, R. B., and Hackett, P. B. (1985). Characterization of ribosome binding on Rous sarcoma virus RNA *in vitro*. *J. Virol.* **56,** 683–690.

Philipson, L., Andersson, P., Olshevsky, U., Weinberg, R., Baltimore, D., and Gesteland, R. (1978). Translation of MuLV and MSV RNAs in nuclease-treated reticulocyte extracts: Enhancement of the *gag–pol* polypeptide with yeast suppressor tRNA. *Cell* **13,** 189–199.

Privalsky, M. L., and Bishop, J. M. (1982). Proteins specified by avian erythroblastosis virus: Coding region localization and identification of a previously undetected *erb*-B polypeptide. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 3958–3962.

Racaniello, V. R., and Baltimore, D. (1981). Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. *Proc. Natl. Acad. Sci. U.S.A.* **78,** 4887–4891.

Raghow, R., and Granoff, A. (1983). Cell-free translation of frog virus 3 mRNAs. *J. Biol. Chem.* **258,** 571–578.

Read, G. S., and Frenkel, N. (1983). Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides. *J. Virol.* **46,** 498–512.

Reddy, V. B., Thimmappaya, B., Dhar, R., Subramanian, K. N., Zain, B. S., Pan, J., Ghosh, P. K., Celma, M. L., and Weissman, S. M. (1978). The genome of simian virus 40. *Science* **200,** 494–502.

Reichel, P. A., Merrick, W. C., Siekierka, J., and Mathews, M. B. (1985). Regulation of a protein synthesis initiation factor by adenovirus VA-RNA$_I$. *Nature (London)* **313,** 196–200.

Rice, A. P., and Kerr, I. M. (1984). Interferon-mediated, doubled-stranded RNA-dependent protein kinase is inhibited in extracts from vaccinia virus-infected cells. *J. Virol.* **50,** 229–236.

Rice, A. P., and Roberts, B. E. (1983). Vaccinia virus induces cellular mRNA degradation. *J. Virol.* **47,** 529–539.

Rice, A. P., Duncan, R., Hershey, J. W. B., and Kerr, I. M. (1985). Double-stranded RNA-dependent protein kinase and 2-5A system are both activated in interferon-treated, EMC virus-infected HeLa cells. *J. Virol.* **54,** 894–898.

Rice, C. M., Lenches, E. M., Eddy, S. R., Shin, S. J., Sheets, R. L., and Strauss, J. H. (1985). Nucleotide sequence of yellow fever virus: Implications for flavivirus gene expression and evolution. *Science* **229,** 726–735.

Rice, C. M., Strauss, E. G., and Strauss, J. H. (1986). Structure of the flavivirus genome. *In* "The Togaviruses and Flaviviruses" (S. Schlesinger and M. Schlesinger, eds.). Plenum, New York, in press.

Robertson, J. S. (1979). 5′ and 3′ terminal nucleotide sequences of the RNA genome segments of influenza virus. *Nucleic Acids Res.* **6,** 3745–3757.

Robinson, M., Lilley, R., Little, S., Emtage, J. S., Yarranton, G., Stephens, P., Millican, A., Eaton, M., and Humphreys, G. (1984). Codon usage can affect efficiency of translation of genes in *E. coli*. *Nucleic Acids Res.* **12,** 6663–6671.

Rose, J. K., Trachsel, H., Leong, K., and Baltimore, D. (1978). Inhibition of translation by poliovirus: Inactivation of a specific initiation factor. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2732–2736.

Rosel, J., and Moss, B. (1985). Transcriptional and translational mapping and nucleotide sequence analysis of a vaccinia virus gene encoding the precursor of the major core polypeptide 4b. *J. Virol.* **56,** 830–838.

Rosemond-Hornbeak, H., and Moss, B. (1975). Inhibition of host protein synthesis by vaccinia virus: Fate of cell mRNA and synthesis of small poly(A)-rich polyribonucleotides in the presence of actinomycin D. *J. Virol.* **16,** 34–42.

Rosen, H., Knoller, S., and Kaempfer, R. (1981). Messenger RNA specificity in the inhibition of eukaryotic translation by double-stranded RNA. *Biochemistry* **20,** 3011–3020.

Rosen, C., Sodroski, J., Chun-Goh, W., Dayton, A., Lippke, J., and Haseltine, W. A. (1986). Post-transcriptional regulation accounts for the transactivation of the human T-lymphotropic virus type III. *Nature (London)* **319,** 555–559.

Saborio, J. L., Pong, S.-S., and Koch, G. (1974). Selective and reversible inhibition of initiation of protein synthesis in mammalian cells. *J. Mol. Biol.* **85,** 195–211.

Safer, B. (1983). 2B or not 2B: Regulation of the catalytic utilization of eIF2. *Cell* **33,** 7–8.

Salzman, N. P., Shatkin, A. J., and Sebring, E. D. (1964). The synthesis of a DNA-like RNA in the cytoplasm of HeLa cells infected with vaccinia virus. *J. Mol. Biol.* **8,** 405–416.

Samuel, C. E., Duncan, R., Knutson, G. S., and Hershey, J. W. B. (1984). Mechanisms of interferon action. Increased phosphorylation of eIF-2α in interferon-treated, reovirus-infected mouse fibroblasts. *J. Biol. Chem.* **259,** 13451–13457.

Sarkar, G., Pelletier, J., Bassel-Duby, R., Jayasuriya, A., Fields, B. N., and Sonenberg, N. (1985). Identification of a new polypeptide coded by reovirus gene S1. *J. Virol.* **54,** 720–725.

Schek, N., and Bachenheimer, S. L. (1985). Degradation of cellular mRNAs induced by a virion-associated factor during herpes simplex virus infection of Vero cells. *J. Virol.* **55,** 601–610.

Schneider, R. J., Weinberger, C., and Shenk, T. (1984). Adenovirus VA_I RNA facilitates the initiation of translation in virus-infected cells. *Cell* **37,** 291–298.

Schneider, R. J., Safer, B., Munemitsu, S. M., Samuel, C. E., and Shenk, T., (1985). Adenovirus VA_I RNA prevents phosphorylation of eIF2α subsequent to infection. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 4321–4325.

Schwartz, D. E., Tizard, R., and Gilbert, W. (1983). Nucleotide sequence of Rous sarcoma virus. *Cell* **32,** 853–869.

Sen, G. C., Taira, H., and Lengyel, P. (1978). Interferon, double-stranded RNA, and protein phosphorylation. *J. Biol. Chem.* **253,** 5915–5921.

Sharpe, A. H., and Fields, B. N. (1982). Reovirus inhibition of cellular RNA and protein synthesis: Role of the S4 gene. *Virology* **122,** 381–391.

Sharpe, A. H., and Fields, B. N. (1984). *In* "The Reoviridae" (W. K. Joklik, ed.), pp. 431–464. Plenum, New York.

Shatkin, A. J. (1976). Capping of eucaryotic mRNAs. *Cell* **9,** 645–653.

Shatkin, A. J. (1983). Molecular mechanisms of virus-mediated cytopathology. *Philos. Trans. R. Soc. London Ser. B* **303,** 167–176.

Shatkin, A. J. (1985). mRNA cap binding proteins: Essential factors for initiating translation. *Cell* **40,** 223–224.

Shatkin, A. J., and Kozak, M. (1983). *In* "The Reoviridae" (W. K. Joklik, ed.), pp. 79–106. Plenum, New York.

Shaw, M. W., Lamb, R. A., Erickson, B. W., Briedis, D. J., and Choppin, P. W. (1982). Complete nucleotide sequence of the neuraminidase gene of influenza B virus. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 6817–6821.

Shaw, M. W., Choppin, P. W., and Lamb, R. A. (1983). A previously unrecognized B virus glycoprotein from a bicistronic mRNA that also encodes the viral neuraminidase. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 4879–4883.

Sheppard, R. D., Raine, C. S., Bornstein, M. B., and Udem, S. A. (1985). Measles virus matrix protein synthesized in a subacute sclerosing panencephalitis cell line. *Science* **228,** 1219–1221.

Shih, D. S., and Kaesberg, P. (1973). Translation of brome mosaic viral ribonucleic acid in a cell-free system derived from wheat embryo. *Proc. Natl. Acad. Sci. U.S.A.* **70,** 1799–1803.

Shinnick, T. M., Lerner, R. A., and Sutcliffe, J. G. (1981). Nucleotide sequence of Moloney murine leukaemia virus. *Nature (London)* **293,** 543–548.

Siekierka, J., Manne, V., and Ochoa, S. (1984). Mechanism of translational control by partial phosphorylation of the α subunit of eukaryotic initiation factor 2. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 352–356.

Siekierka, J., Mariano, T. M., Reichel, P. A., and Mathews, M. B. (1985). Translational control by adenovirus: Lack of VA-RNA$_I$ during adenovirus infection results in phosphorylation of eIF-2 and inhibition of protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 1959–1963.

Silverstein, S., and Engelhardt, D. L. (1979). Alterations in the protein synthetic apparatus of cells infected with herpes simplex virus. *Virology* **95,** 334–342.

Simizu, B. (1984). *In* "Comprehensive Virology" (H. Fraenkel-Conrat and R. R. Wagner, eds.), Vol. 19, pp. 465–499. Plenum, New York.

Simon, L. D., Randolph, B., Irwin, N., and Binkowski, G. (1983). Stabilization of proteins by a bacteriophage T4 gene cloned in *E. coli*. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 2059–2062.

Skinner, M. A., and Siddell, S. G. (1985). Coding sequence of coronavirus MHV-JHM mRNA 4. *J. Gen. Virol.* **66,** 593–596.

Skinner, M. A., Ebner, D., and Siddell, S. G. (1985). Coronavirus MHV-JHM mRNA 5 has a sequence arrangement which potentially allows translation of a second, downstream open reading frame. *J. Gen. Virol.* **66,** 581–592.

Skup, D., and Millward, S. (1980). Reovirus-induced modification of cap-dependent translation in infected L cells. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 152–156.

Skup, D., Zarbl, H., and Millward, S. (1981). Regulation of translation in L-cells infected with reovirus. *J. Mol. Biol.* **151,** 35–55.

Smith, A. E. (1977). *In* "Gene Expression" (B. F. C. Clark *et al.*, eds.). *Fed. Eur. Biol. Soc. Symp.* **43,** 37–46.

Smith, A. E., and Marcker, K. A. (1970). Cytoplasmic methionine transfer RNAs from eukaryotes. *Nature (London)* **226,** 607–610.

Smith, A. E., Smith, R., and Paucha, E. (1978). Extraction and fingerprint of SV40 large and small T-antigens. *J. Virol.* **28,** 140–153.

Smith, G. E., Summers, M. D., and Fraser, M. J. (1983). Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Mol. Cell. Biol.* **3,** 2156–2165.

Sonenberg, N., Trachsel, H., Hecht, S., and Shatkin, A. J. (1980). Differential stimulation of capped mRNA translation *in vitro* by cap binding protein. *Nature (London)* **285,** 331–333.

Sonenberg, N., Guertin, D., Cleveland, D., and Trachsel, H. (1981). Probing the function of the eucaryotic 5′ cap structure by using a monoclonal antibody directed against cap-binding proteins. *Cell* **27,** 563–572.

Spaan, W., Delius, H., Skinner, M., Armstrong, J., Rottier, P., Smeekens, S., van der Zeijst, B., and Siddell, S. G. (1983). Coronavirus mRNA synthesis involves fusion of non-contiguous sequences. *EMBO J.* **2,** 1839–1844.

Spena, A., Krause, E., and Dobberstein, B. (1985). Translation efficiency of zein mRNA is reduced by hybrid formation between the 5′- and 3′-untranslated region. *EMBO J.* **4,** 2153–2158.

Spieth, J., Denison, K., Zucker, E., and Blumenthal, T. (1985). The nucleotide sequence of a nematode vitellogenin gene. *Nucleic Acids Res.* **13,** 7129–7138.

Spindler, K. R., and Berk, A. J. (1984a). Translation efficiency of adenovirus early region 1A mRNAs deleted in the 5′ untranslated region. *J. Virol.* **51,** 884–887.

Spindler, K. R., and Berk, A. J. (1984b). Rapid intracellular turnover of adenovirus 5 early region 1A proteins. *J. Virol.* **52,** 706–710.

Stenberg, R. M., and Pizer, L. I. (1982). Herpes simplex virus-induced changes in cellular and adenovirus RNA metabolism in an adenovirus type 5-transformed human cell line. *J. Virol.* **42,** 474–487.

Stevely, W. S., and McGrath, B. M. (1978). The effect of hypertonic conditions on protein synthesis in cells infected with herpes virus. *FEBS Lett.* **87,** 308–310.

Strauss, E. G., Rice, C. M., and Strauss, J. H. (1983). Sequence coding for the alphavirus nonstructural proteins is interrupted by an opal termination codon. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 5271–5275.

Svensson, C., and Akusjarvi, G. (1984). Adenovirus VA RNA_I: A positive regulator of mRNA translation. *Mol. Cell. Biol.* **4,** 736–742.

Svensson, C., and Akusjarvi, G. (1985). Adenovirus VA RNA_I mediates a translational stimulation which is not restricted to the viral mRNAs. *EMBO J.* **4,** 957–964.

Svensson, C., Pettersson, U., and Akusjarvi, G. (1983). Splicing of adenovirus 2 early region 1A mRNAs is non-sequential. *J. Mol. Biol.* **165,** 475–499.

Svitkin, Y. V., Ginevskaya, V. A., Ugarova, T. Y., and Agol. V. I. (1978). A cell-free model of the EMC virus-induced inhibition of host cell protein synthesis. *Virology* **87,** 199–203.

Tahara, S. M., Morgan, M. A., and Shatkin, A. J. (1981). Two forms of purified m^7G-cap binding protein with different effects on capped mRNA translation in extracts of uninfected and poliovirus-infected HeLa cells. *J. Biol. Chem.* **256,** 7691–7694.

Tarpley, W. G., and Temin, H. M. (1984). The location of v-*src* in a retrovirus vector determines whether the virus is toxic or transforming. *Mol. Cell. Biol.* **4,** 2653–2660.

Thimmappaya, B., Weinberger, C., Schneider, R. J., and Shenk, T. (1982). Adenovirus VA_I RNA is required for efficient translation of viral mRNAs at late times after infection. *Cell* **31,** 543–551.

Tiollais, P., Pourcel, C., and Dejean, A. (1985). The hepatitis B virus. *Nature (London)* **317,** 489–495.

Toyoda, H., Kohara, M., Kataoka, Y., Suganuma, T., Omata, T., Imura, N., and Nomoto, A. (1984). Complete nucleotide sequences of all three poliovirus serotype genomes. *J. Mol. Biol.* **174,** 561–585.

Tso, J. Y., Sun, X.-H., Kao, T., Reece, K., and Wu, R. (1985). Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. *Nucleic Acids. Res.* **13,** 2485–2502.

Van Steeg, H., Thomas, A., Verbeek, S., Kasperaitis, M., Voorma, H. O., and Benne, R. (1981). Shutoff of neuroblastoma cell protein synthesis by Semliki Forest virus: Loss of ability of crude initiation factors to recognize early SFV and host mRNAs. *J. Virol.* **38,** 728–736.

Van Steeg, H., Kasperaitis, M., Voorma, H. O., and Benne, R. (1984). Infection of neuroblastoma cells by SFV. *Eur. J. Biochem.* **138,** 473–478.

Varenne, S., Buc, J., Lloubes, R., and Lazdunski, C. (1984). Translation is a nonuniform process. Effects of tRNA availability on the rate of elongation of nascent polypeptide chains. *J. Mol. Biol.* **180,** 549–576.

Vassef, A., Ben-Hamida, F., Dru, A., and Beaud, G. (1982). Translational control of early protein synthesis at the late stage of vaccinia virus infection. *Virology* **118,** 45–53.

Villarreal, L. P., White, R. T., and Berg, P. (1979). Mutational alterations within the SV40 leader segment generate altered 16S and 19S mRNAs. *J. Virol.* **29,** 209–219.

Wagner, E. K. (1985). Individual HSV transcripts: Characterization of specific genes. *In* "The Herpesviruses" (B. Roizman, ed.), Vol. 3, pp. 45–104. Plenum, New York.

Wagner, M. J., Sharp, J. A., and Summers, W. C. (1981). Nucleotide sequence of the thymidine kinase gene of herpes simplex virus type 1. *Proc. Natl. Acad. Sci. U.S.A.* **78,** 1441–1445.

Walden, W. E., Godefroy-Colburn, T., and Thach, R. E. (1981). The role of mRNA competition in regulating translation. *J. Biol. Chem.* **256,** 11739–11746.

Weiner, A. M., and Weber, K. (1973). A single UGA codon functions as a natural termination signal in the coliphage Qβ coat protein cistron. *J. Mol. Biol.* **80,** 837–855.

Welch, W. J., and Feramisco, J. R. (1985). Disruption of the three cytoskeletal networks

in mammalian cells does not affect transcription, translation, or protein translocation changes induced by heat shock. *Mol. Cell. Biol.* **5,** 1571–1581.

Wengler, G., and Wengler, G. (1976). Protein synthesis in BHK-21 cells infected with Semliki Forest virus. *J. Virol.* **17,** 10–19.

Whitaker-Dowling, P., and Youngner, J. S. (1984). Characterization of a specific kinase inhibitory factor produced by vaccinia virus which inhibits the interferon-induced protein kinase. *Virology* **137,** 171–181.

Willis, D., Foglesong, D., and Granoff, A. (1984). Nucleotide sequence of an immediate-early frog virus 3 gene. *J. Virol.* **52,** 905–912.

Willis, D. B., Goorha, R., and Chinchar, V. G. (1985). Macromolecular synthesis in cells infected by frog virus 3. *Curr. Top. Microbiol. Immunol.* **116,** 77–106.

Willumsen, B. M., Ellis, R. W., Scolnick, E. M., and Lowy, D. R. (1984). Further genetic localization of the transforming sequences of the p21 v-*ras* gene of Harvey murine sarcoma virus. *J. Virol.* **49,** 601–603.

Wilson, M. C., and Darnell, J. E. (1981). Control of mRNA concentration by differential cytoplasmic half-life. *J. Mol. Biol.* **148,** 231–251.

Wold, W. S. M., Deutscher, S. L., Takemori, N., Bhat, B. M., and Magie, S. C. (1986). Evidence that AGUAUAUGA and CCAAGAUGA initiate translation in the same mRNA in region E3 of adenovirus. *Virology* **148,** 168–180.

Wong, S. T., Mastropaolo, W., and Henshaw, E. C. (1982). Differential phosphorylation of soluble versus ribosome-bound eIF2 in the Ehrlich ascites tumor cell. *J. Biol. Chem.* **257,** 5231–5238.

Yates, J. R., and Nuss, D. L. (1982). Resistance to inhibitors of mammalian cell protein synthesis induced by preincubation in hypertonic growth medium. *J. Biol. Chem.* **257,** 15030–15034.

Yoshinaka, Y., Katoh, I., Copeland, T. D., and Oroszlan, S. (1985a). Murine leukemia virus protease is encoded by the *gag–pol* gene and is synthesized through suppression of an amber termination codon. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 1618–1622.

Yoshinaka, Y., Katoh, I., Copeland, T. D., and Oroszlan, S. (1985b). Translational read-through of an amber termination codon during synthesis of feline leukemia virus protease. *J. Virol.* **55,** 870–873.

Ziff, E. B. (1985). Splicing in adenovirus and other animal viruses. *Int. Rev. Cytol.* **93,** 327–358.

Zitomer, R. S., Walthall, D. A., Rymond, B. C., and Hollenberg, C. P. (1984). *S. cerevisiae* ribosomes recognize non-AUG initiation codons. *Mol. Cell. Biol.* **4,** 1191–1197.

Zweerink, H. J., and Joklik, W. K. (1970). Studies on the intracellular synthesis of reovirus-specified proteins. *Virology* **41,** 501–518.