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Cytopathogenesis and Inhibition of Host Gene Expression by RNA Viruses

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INTRODUCTION

Some viruses replicate in cell cultures without causing any obvious pathological changes in their host cells. The more usual case, however, is that virus replication interferes with normal host cell function in ways that are harmful or pathological. Morphological changes may include cell rounding, cell lysis, cell fusion to form syncytia, or more subtle changes in cell shape. Such morphological changes are usually referred to as cytopathic effects (CPE). In addition, many viruses inhibit host cell gene expression, which plays a major role in the ability of these viruses to cause disease. The term "cytopathogenesis," or pathogenesis at the cellular level, is meant to be broader than CPE and includes other cellular changes that contribute to viral pathogenesis in addition to changes that are visible at the microscopic level. The goal of this review is to place recent work on the inhibition of host gene expression by RNA viruses in the context of the pathogenesis of virus infections.

Members of many different virus families inhibit the expression of host genes during the process of virus replication. The typical explanation for this effect given in textbooks is that it provides higher levels of cellular resources such as nucleoside triphosphates to be used for biosynthesis of viral gene products (see, e.g., reference 73). However, there are many examples of viral mutants that are defective in their ability to inhibit host gene expression yet replicate as well as wild-type viruses in most cell types. The cell types that restrict the growth of such mutants compared to wild-type viruses are often those that can mount a vigorous antiviral defense. Such observations support the idea that the role of the virus-induced inhibition of host gene expression is to inhibit the host antiviral response. This idea is not new. Some of the earliest papers that describe the virus-induced inhibition of host RNA and protein synthesis relate these effects to the inhibition of the host cell interferon response (see, e.g., references 132 and 139). The argument will be made in this review that the ability to inhibit the host antiviral response through the inhibition of host gene expression is a critical aspect of viral pathogenesis.

The principle that viruses may inhibit host gene expression in order to inhibit the antiviral defense of the host is pretty straightforward. However, sorting out the relationship between viral cytopathogenesis and the host antiviral response can be difficult in practice. For some viruses, phenomena once considered CPE of the virus are now known to be part of the antiviral response of the host (57). For example, the inhibition of host protein synthesis in virus-infected cells is often considered a direct effect of viral gene products. Indeed, there are cases, such as most picornaviruses, in which viral gene products act directly to inhibit host protein synthesis without affecting

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viral protein synthesis. However, in many cases the inhibition of host protein synthesis results from activation of the host response to viral double-stranded RNA (dsRNA), such as activation of protein kinase R (PKR). The induction of apoptosis by viruses is another example of what would appear to be a CPE of the virus but is most likely to be an antiviral response of the host (115). Cell death by apoptosis is now widely considered to be a host response to limit virus replication through elimination of virus-infected cells. Some viruses appear to have adapted to growth in apoptotic cells. For example, some influenza virus and Sindbis virus strains replicate less efficiently in cells that overexpress the antiapoptotic protein Bcl-2 than in control cells that undergo apoptosis more rapidly (100, 129). However, in general, inhibition of apoptosis prolongs the period of virus replication and leads to higher virus yields.

This review will discuss the molecular mechanisms involved in virus-induced inhibition of each step in host gene expression—transcription, RNA processing and transport, and translation—with the goal of distinguishing the direct effects of viral gene products from the indirect effects of the host antiviral response. The review will focus on members of three different RNA virus families, picornaviruses, influenza viruses, and rhabdoviruses. These examples were chosen because viral gene products responsible for inhibiting host gene expression have been identified, as have some of the molecular targets of the host. These viruses were also chosen because there are interesting parallels in their inhibition of host gene expression, despite the diversity of their strategies of viral gene expression. Finally, an attempt will be made to relate viral cytopathogenesis in cell cultures to viral tropism and pathogenesis in intact organisms. It is widely recognized that the ability of viruses to induce CPE in culture bears little relationship to the ability to cause disease in intact organisms. However, the factors that influence the balance of viral cytopathogenesis and the host antiviral response apparent in cell cultures are likely to be major determinants of the ability of viruses to cause disease in animal hosts.

INHIBITION OF HOST TRANSCRIPTON

The host transcriptional apparatus represents a logical target for inhibition by most RNA viruses, since these viruses replicate in the cytoplasm of the host cell without any requirement for host transcriptional activity. Influenza viruses are an obvious exception, since they replicate in the nucleus of the host cell and require newly synthesized host transcripts for generation of the capped oligonucleotides used as primers for viral mRNA synthesis. For many members of other RNA virus families, such as picornaviruses and rhabdoviruses, the inhibition of host RNA synthesis is a prominent feature of virus infection that has been recognized for many years (6, 61, 132, 139, 148). For poliovirus, the prototype picornavirus, and vesicular stomatitis virus (VSV), the prototype rhabdovirus, substantial progress has been made in identifying the viral gene products responsible for the inhibition of host transcription and their molecular targets in the host cell. There are striking parallels between these two viruses. Neither virus encodes a protein whose sole function is to inhibit host gene expression. Instead, viral proteins that play important roles in the virus replicative cycle, the poliovirus 3C protease and the VSV matrix (M) protein, serve a second function in the inhibition of host gene expression. For both viruses, most viral proteins are located in the cytoplasm of infected cells, where virus replication and assembly take place. However, the poliovirus 3C protease (as well as its precursor, 3CD) and the VSV M protein are located in both the cytoplasm and nucleus of infected cells

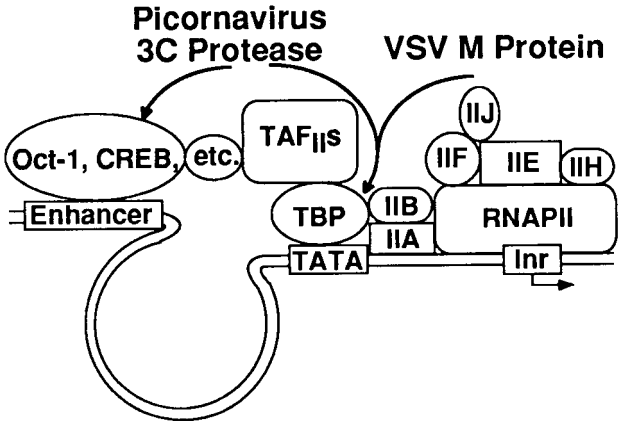

FIG. 1. Formation of preinitiation complex for RNAPII. Enhancer-binding proteins such as Oct-1 and CREB interact with TAF_{II} subunits of TFIID. The TBP subunit of TFIID binds the TATA box DNA element and recruits the remaining basal transcription factors, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, and TFIIJ, as well as RNAPII, to the promoter initiation site (Inr). Proteins which are targets for inhibition by picornavirus 3C protease and VSV M protein are indicated.

(38, 87), where they perform their separate functions in virus replication and inhibition of host gene expression, respectively. Both of these viral proteins inhibit transcription by all three host RNA polymerases (RNAP). Also, in the case of host RNAPII, the inhibition is caused by inactivation of the same transcription initiation factor, TFIID.

Viral Inhibition of TFIID Activity

TFIID is one of a set of seven initiation factors required in addition to RNAPII for transcription from RNAPII-dependent promoters (Fig. 1) (113). These factors are usually referred to as general transcription factors, because of their requirement for all RNAPII-dependent genes, or as basal transcription factors, because there is relatively little transcription from RNAPII-dependent promoters in the absence of other proteins that bind DNA sequence-specific enhancer elements. Such enhancer elements vary widely among different genes and form the basis for the differential control of gene expression. TFIID is a multisubunit complex consisting of a DNA-binding subunit, TATA box-binding protein (TBP), and a set of TBP-associated factors (TAFs) (18). TFIID is the first basal transcription factor assembled onto RNAPII-dependent promoters through binding of TBP to the TATA box DNA sequence located upstream of most promoters. Even promoters that lack a TATA box require TFIID for transcription initiation, but in these cases TFIID is recruited to the promoter by protein-protein interaction with other transcription factors that bind to TATA-independent promoters. TBP is the only subunit of TFIID required for basal transcription in vitro. However, activation of transcription by proteins that bind DNA sequence-specific enhancer elements requires interaction with one or more TAF subunits either directly or indirectly through so-called adapter proteins. Because of its central role in both basal and activated transcription, TFIID is an obvious candidate as a target for inactivation by viruses that inhibit the general expression of host genes.

The same experimental approach was used to demonstrate that TFIID is inactive in cells infected both with poliovirus and with VSV. Earlier experiments had shown that the inhibition of transcription occurs at the initiation step rather than elon-

gation and that the activity of RNAPII is not affected by virus infection (29, 137). These results suggested that one or more of the transcription initiation factors for RNAPII is inactivated. Furthermore, the inhibition of RNAPII-dependent transcription does not depend on the nature of the promoter (86), suggesting that the target for inactivation is a basal transcription factor. Nuclear extracts capable of initiating RNAPII-dependent transcription in vitro were used to demonstrate that TFIID, either isolated from uninfected cells or in the form of purified recombinant TBP, could overcome the virus-induced inhibition of transcription in nuclear extracts from infected cells. Conversely, TFIID isolated from infected cells was not able to initiate transcription in the presence of other transcription factors and RNAPII from uninfected cells (72, 144, 147). These experiments clearly established TFIID as the major target for the virus-induced inhibition of transcription by RNAPII. The mechanism of the inactivation of TFIID is of considerable interest because of the implications for viral pathogenesis, as well as the idea that something new about the regulation of TFIID activity will be learned.

Viral Proteins Responsible for Inhibition of Host TFIID

The viral gene product responsible for inactivating TFIID in poliovirus is the viral 3C protease. This was shown by in vitro transcription experiments in which the activity of nuclear extracts or purified TBP was inhibited by treatment with purified recombinant 3C protease (26, 144) and also by in vivo experiments in which 3C protease expressed in transfected cells in the absence of other viral components was able to inhibit the expression of cotransfected target genes (144). The poliovirus 3C protease normally functions in the cleavage of the P1, P2, and P3 precursor proteins. These proteins are generated by initial cleavage of the poliovirus polyprotein by another viral protease, 2A (107). Despite the expression of two viral proteases, there are relatively few host proteins that are cleaved in poliovirus-infected cells (130). The specificity of 3C protease is for cleavage between Gln-Gly bonds that are in the proper sequence and structural context in the viral polyproteins. There are three Gln-Gly bonds in the 300-amino-acid (aa) sequence of human TBP. The major cleavage product of TBP results from cleavage by 3C protease at position 18, and the inhibition of RNAPII-dependent transcription was originally attributed to cleavage at this site (30). However, deletion of the N-terminal region of TBP, including deletion of the amino-terminal 18 aa, has little if any effect on its activity in basal transcription (105, 144). This result is difficult to reconcile with the dramatic inhibition of TBP activity resulting from treatment with 3C protease in vitro under conditions that produce the TBP cleavage product lacking the N-terminal 18 aa. An effect of 3C that is independent of its proteolytic activity appears to be ruled out by absence of inhibitory effect of a 3C mutant lacking proteolytic activity (144). One possibility is that there is a secondary cleavage at the Gln-Gly bond at position 108 of TBP that is responsible for the observed inhibition (144). Alternatively, the TBP cleavage product lacking the N-terminal 18 aa may be susceptible to as yet undiscovered inhibitory mechanisms that do not affect intact TBP.

In VSV and closely related rhabdoviruses, the viral gene product responsible for inhibition of host transcription is the viral M protein (2, 9, 10, 39, 102, 127). M protein plays a major role in virus assembly by binding the viral nucleocapsid to the cytoplasmic surface of the host plasma membrane and inducing the budding process that generates the viral envelope (77). Unlike poliovirus 3C protease, M protein does not have any known catalytic activity that could be responsible for the inhibition of host gene expression. Therefore, it was not expected that a viral protein normally considered to play a structural role in the virion would also be responsible for inhibiting host gene expression. There are two arguments supporting a role of M protein in inhibition of host gene expression. First, M protein expressed in transfected cells in the absence of other viral components inhibits the transcription of cotransfected target genes (2, 9, 10, 39, 102, 127), and second, two different VSV mutants have been independently isolated that are defective in the inhibition of host transcription and contain the same point mutation in their M proteins (28, 39). This mutation results in substitution of arginine for the methionine at position 51 (M51R mutation) of the 229-aa M protein. The M51R mutant M protein is 10- to 100-fold less active than is the wild-type M protein in the inhibition of host-directed transcription in cotransfection experiments (3, 10, 39). An additional M protein mutation, which results in a two- to three-fold reduction in inhibitory activity, has been identified (N163D) in viruses isolated from persistently infected cells (3). The ability of M protein to inhibit host gene expression is genetically separate from its role in virus assembly. Both the M51R and N163D mutant M proteins are defective in the inhibition of host gene expression yet function as effectively as wild-type M protein in virus assembly. In contrast, deletion mutants of M protein lacking the N-terminal 20 to 40 aa have the complementary phenotype. They are as effective as wild-type M protein in the inhibition of host gene expression yet cannot function in virus assembly (10, 102). Thus, the ability of M protein to inhibit host gene expression represents a function independent of its role in virus assembly.

The VSV M protein is a very potent inhibitor of host transcription. It has been estimated that 10,000 copies of M protein per cell result in 50% inhibition of host RNAPII-dependent transcription in transfection experiments (86). This is approximately 1,000-fold less than the number of copies of M protein expressed in VSV-infected cells. This potency probably accounts for earlier data indicating that M protein was not involved in the inhibition of host gene expression. For example, temperature-sensitive M protein mutants usually inhibit host gene expression at the nonpermissive temperature for virus assembly (136), but this is due to the production of a small amount of functional M protein even at the nonpermissive temperature (86). The potency of M protein in the inhibition of host transcription also accounts for the difficulty in expressing detectable amounts of M protein from recombinant vectors that depend on host RNAPII activity (12, 79), since expression of a small amount of M protein from these vectors inhibits the further synthesis of M mRNA (9). Despite the potency of M protein in vivo, it has not been possible to inhibit the transcriptional activity of TFIID in nuclear extracts from uninfected cells by the addition of exogenous M protein in vitro, as can be done with the poliovirus 3C protease (H. Yuan and D. S. Lyles, unpublished data). Thus, M protein may inactivate RNAPII-dependent transcription by binding to TFIID in vivo by a mechanism that cannot be re-created in vitro in nuclear extracts. However, attempts to coimmunoprecipitate M protein and TBP with antibodies to either protein have yielded negative results (Yuan and Lyles, unpublished). Alternatively, M protein may act indirectly to inactivate TFIID in vivo by activating an inhibitory host factor. Such a host factor might be an inhibitory subunit of TFIID that has yet to be discovered or a novel posttranslational modification of TFIID that inhibits its activity.

Other Molecular Targets for Inhibition of Host Transcription

Finally, it must be considered that more than one mechanism may be involved in the virus-induced inhibition of host transcription. First, more than one molecular target may be inactivated, and second, other viral gene products may participate in the inhibition of host transcription. The poliovirus 3C protease has been shown to cleave and inactivate two different DNA sequence-specific enhancer-binding proteins, Oct-1 and cyclic AMP-responsive element-binding protein (CREB), in addition to TBP, so that inhibition of expression of genes activated by these proteins may have multiple inhibitory mechanisms (143, 145). The 3C proteases of other picornaviruses may cleave additional targets that are not recognized by the poliovirus enzyme. For example, the 3C protease of foot-and-mouth disease virus (FMDV) cleaves histone H3, which would have an indirect effect on host transcription by altering the structure of the chromatin template. Histone cleavage has not been observed with other picornaviruses, and so this mechanism of transcription inhibition may be specific for FMDV.

Both the poliovirus 3C protease and the VSV M protein inhibit the activity of all three host RNA polymerases (2, 25, 26, 116). The molecular target involved in the inhibition of RNAPI-dependent transcription has not been identified. In RNAPIII-dependent transcription, both the poliovirus 3C protease and the VSV M protein inactivate the same transcription factor, TFIIIC (24, 25, 42) (Yuan and Lyles, unpublished).

Other viral gene products besides poliovirus 3C protease and VSV M protein probably contribute to the inhibition of host transcription. Both the 3C protease and the 2A protease of poliovirus inhibit host transcription when expressed in transfected cells in the absence of other viral gene products (31, 144). However, in contrast to 3C protease, addition of 2A protease to nuclear extracts from uninfected cells does not inhibit RNAPII-dependent transcription, even though TBP is cleaved by 2A protease at the single Tyr-Gly bond at position 34 (142). Thus, the inhibition of transcription by 2A protease may be an indirect effect not related to cleavage of TBP. In VSV, none of the other viral gene products besides M protein inhibit host transcription when expressed in the absence of other viral gene products (39). However, there is considerable evidence that the viral leader RNA can inhibit host transcription and therefore may act together with M protein in the inhibition of host transcription in VSV-infected cells. Leader RNA is a 45- to 50-nucleotide noncoding RNA transcribed from the 3′ end of the viral genome by the viral RNA polymerase prior to transcription of the viral mRNAs. Even though leader RNA alone cannot inhibit host transcription in vivo (35, 106), leader RNA or DNA oligonucleotides with leader sequence can inhibit host transcription by RNAPII and RNAPIII in vitro (54, 55, 91). The molecular target of inhibition by leader RNA has not been identified, but the results of initial experiments indicate that it has an apparent molecular mass of 65 kDa, which is too small to be TFIID, which has a molecular mass of >300 kDa (54). Thus, there may be multiple molecular targets involved in the inhibition of host transcription in VSV-infected cells as well as in poliovirus-infected cells.

INHIBITION OF PROCESSING AND TRANSPORT OF HOST RNA

Inhibition of host gene expression at the posttranscriptional level is another logical strategy for RNA viruses to prevent an antiviral response in the host, since many processes are required for expression of host genes that are not required for expression of viral genes. Inhibition of processing and transport of host RNA is a prominent feature of influenza virus infection, in which a single viral protein, NS1, inhibits multiple steps required for expression of host genes (21). Likewise, in VSV-infected cells, multiple RNA processing events are inhibited, and this inhibition has recently been attributed to the activity of the M protein (60). These two viruses provide interesting parallels that illustrate some of the general principles of the inhibition of host gene expression. However, differences in the details of how they accomplish this task reflect the relative dependence of these two viruses on host processes for the expression of viral genes.

Multiple Functions of Viral Inhibitory Proteins

The first principle is that RNA viruses inhibit host gene expression at multiple steps. The VSV M protein inhibits the initiation of transcription of host genes, as discussed in the preceding section, and also inhibits the transport of host RNAs and proteins between the nucleus and the cytoplasm (60). The influenza virus NS1 protein inhibits at least two steps in the processing of the 3′ ends of host mRNAs and also inhibits the splicing of host mRNAs (21, 41, 83, 97, 112). The inhibition of multiple processes probably reflects the fact that no single inhibitory mechanism is completely effective. A corollary of this principle is that different inhibitory mechanisms will assume greater or lesser prominence depending on the type of host cell that is infected and the time postinfection that the inhibition occurs.

The second principle, which follows from the first, is that viral proteins involved in the inhibition of host gene expression are multifunctional. Both the influenza virus NS1 protein and the VSV M protein are dramatic examples. The different activities of NS1 protein are, to some extent, divided between two different protein domains, an approximately 80-aa N-terminal RNA-binding domain and a C-terminal domain of about 140 aa, the 'activation domain,' involved in protein-protein interactions with host molecular targets (90, 109, 110); C. Y. Chien, R. Tejero, Y. Huang, D. E. Zimmerman, C. B. Rios, R. M. Krug, and G. T. Montelione, Letter, Nat. Struct. Biol. **4:**891–895, 1997; J. Liu, P. A. Lynch, C. Y. Chien, G. T. Montelione, R. M. Krug, and H. M. Berman, Letter, Nat. Struct. Biol. **4:**896–899, 1997). The activities of both domains are remarkably diverse. The RNA-binding domain has a single RNA-binding site (Chien et al., Letter; Liu et al., Letter) but has multiple specificities that are biologically important. NS1 protein binds a specific stem-bulge in the U6 small nuclear RNA (snRNA) involved in most mRNA splicing events (112), as well as a highly divergent sequence in the minor snRNA U6atac (134). NS1 protein also binds to the poly(A) sequence at the 3′ ends of host mRNAs (111). It was originally thought that this binding was involved in the retention of host mRNAs in the nucleus of infected cells, although other mechanisms may account for this effect of NS1 protein, as discussed below. Finally, NS1 protein binds dsRNA with little if any sequence specificity (58, 84). While the ability to bind dsRNA is not directly related to the inhibition of host RNA processing and transport, it is a critical aspect of the inhibition of the host antiviral response since it interferes with the host response to viral dsRNA, such as the activation of protein kinase R. The activation domain of NS1 protein also exhibits multiple binding specificities and contains nonoverlapping binding sites for two different host proteins involved in processing the 3′ ends of host mRNAs (21).

The multiple functions of the VSV M protein in virus assembly as well as in the inhibition of host transcription were

described in the previous section. Unlike for NS1 protein, however, it has not been possible to assign different functions to specific domains. The 229-aa M protein appears to be divided into an N-terminal region of about 50 aa that is highly exposed, as shown by protease susceptibility, and a compactly folded C-terminal region of the remaining 180 aa (69). Most of the N-terminal region to approximately position 40 is dispensable for the function of M protein in inhibition of host gene expression but is required for virus assembly (10, 102). Many mutations throughout the C-terminal region lead to misfolding of M protein and inhibition of both functions (94, 102). While mutations such as N-terminal deletions and the point mutations M51R and N163D separate the virus assembly functions of M protein from its ability to inhibit host gene expression (10), thus far there are no mutations that separate the ability of M protein to inhibit host transcription from its ability to inhibit transport of RNAs and proteins between the nucleus and the cytoplasm. This raises the question whether these two effects of M protein on host gene expression are related by cause and effect. For example, it was proposed that the inhibition of host transcription by M protein was a secondary effect of the inhibition of transport of transcription factors from the cytoplasm to the nucleus (60). This hypothesis was disproved when it was shown that nuclear extracts from infected cells contain normal amounts of TFIID but the TFIID is in an inactive form (147). Nonetheless, it is still a valid hypothesis that the inhibition of transcription and the inhibition of nuclear-cytoplasmic transport may arise from a common cause that has not yet been described, such as a host mechanism which shuts off both processes. Alternatively, M protein may resemble NS1 protein in being able to interact with multiple diverse molecular targets in host cells.

Considerations of cause and effect lead to the next general principle of inhibition of host gene expression. For many RNAs, processing and transport are coupled, so that inhibition of one step leads to inhibition of the other. For example, processing of mRNAs is required to generate proper substrates for transport, so that inhibition of mRNA processing can cause inhibition of transport. In contrast to mRNAs, processing of most snRNAs involves protein factors in the cytoplasm, so that inhibition of transport can cause inhibition of processing. Both viruses under consideration illustrate this principle, although in opposite ways. The VSV M protein appears to inhibit the processing of host RNA by inhibiting nuclear-cytoplasmic transport (60), while the influenza virus NS1 protein appears to inhibit transport of host mRNAs by inhibiting their processing (21). This difference reflects the need for influenza viruses, but not VSV, to transport viral mRNAs and ribonucleoproteins from their site of synthesis in the nucleus to their sites of action in the cytoplasm. Thus, the problem in understanding the effects of NS1 protein is to determine how processing of host RNAs is inhibited while processing of viral RNAs is not.

Inhibition of RNA Processing by Influenza Virus NS1 Protein

The processing events targeted by NS1 protein that are specific to host and not viral mRNAs are those involved in generating polyadenylated 3′ ends (Fig. 2). In infected cells, the 3′ ends of viral mRNAs are generated by the viral RNA polymerase, which is not affected by NS1 protein. Processing of the 3′ ends of host mRNAs occurs in two coupled steps consisting of endonucleolytic cleavage of the mRNA precursor followed by poly(A) addition to the 3′ end of the cleavage product (Fig. 2) (reviewed in reference 27). NS1 protein inhibits both of these steps. It interferes with the cleavage step by binding the 30-kDa subunit of the cleavage and polyadenylation specificity factor (CPSF) (97). CPSF binds to the AAUAAA sequence located 10 to 30 nucleotides upstream of the cleavage site of mRNA precursors and participates with three other cellular factors in the cleavage reaction. Binding of NS1 protein to CPSF does not completely inhibit the cleavage reaction. However, NS1 protein also inhibits the subsequent step by binding an essential factor for polyadenylation. Following cleavage of mRNA precursors, poly(A) polymerase is recruited to the processing complex by CPSF and adds 10 to 12 A residues to the 3′ end. Further elongation by poly(A) polymerase requires a nuclear poly(A)-binding protein (PABII) that is distinct from the poly(A)-binding protein associated with cytoplasmic mRNAs (Fig. 2). NS1 protein binds PABII and interferes with its association with the short poly(A) tails (21). As a result, cellular mRNAs containing 10 to 12 A residues at their 3′ ends accumulate in influenza virus-infected cells and are retained in the nucleus. The reason for the failure to transport these RNAs to the cytoplasm has not been fully established but may be that binding of PABII to the 3′ end of mRNAs is required for transport.

NS1 protein also inhibits splicing of host mRNAs (Fig. 2). Inhibition of splicing may involve binding of the NS1 RNA-binding domain to a specific stem-bulge sequence in the U6 RNA (112). NS1 protein does not interfere with assembly of mRNA precursors into spliceosomes but does interfere with the interaction of U6 with U2 and U4 RNAs necessary for the catalytic steps in splicing (112). One of the effects of blocking splicing at this stage is that the assembly of mRNA precursors into inactive spliceosomes prevents their transport to the cytoplasm, thus contributing to the block in transport of host RNAs in virus-infected cells. This mechanism of inhibition of splicing would have relatively little effect on viral gene expression, since most of the viral proteins are translated from unspliced mRNAs. However, two of the viral genome segments, 7 and 8, encode both spliced and unspliced mRNAs. This includes the mRNA for NS1 protein itself, which is translated from the unspliced mRNA from segment 8. For this reason, splicing cannot be completely inhibited in virus-infected cells. To some extent the mRNAs from segments 7 and 8 are more resistant than host mRNAs to the inhibition of splicing by NS1 protein because they contain *cis*-acting sequences that promote their splicing (83, 120). In the segment 7 mRNA that encodes the M2 protein, this is due to binding of the cellular SF2/ASF alternative splicing factor, which is normally involved in regulation of alternative splicing events (120).

Inhibition of Nuclear-Cytoplasmic Transport by M Protein

Ironically, the block in host RNA processing by influenza virus was first recognized as a block in transport (4) whereas the block in host RNA transport by VSV was first recognized as a block in processing (43, 141). In VSV-infected cells, the processing of snRNAs (43) and rRNAs (141) is rapidly inhibited while the processing of mRNAs and tRNAs is not. This pattern of inhibition reflects the relative sensitivity of processing of the different RNAs to a generalized block in nuclear-cytoplasmic transport of both RNA and proteins that is induced by M protein (60). Because VSV inhibits host transcription, the virus-induced block in RNA transport and processing is most apparent under conditions where the inhibition of transcription is not so pronounced, such as at early times postinfection. The virus-induced inhibition of transcription occurs between 2 and 4 h postinfection, whereas the in-

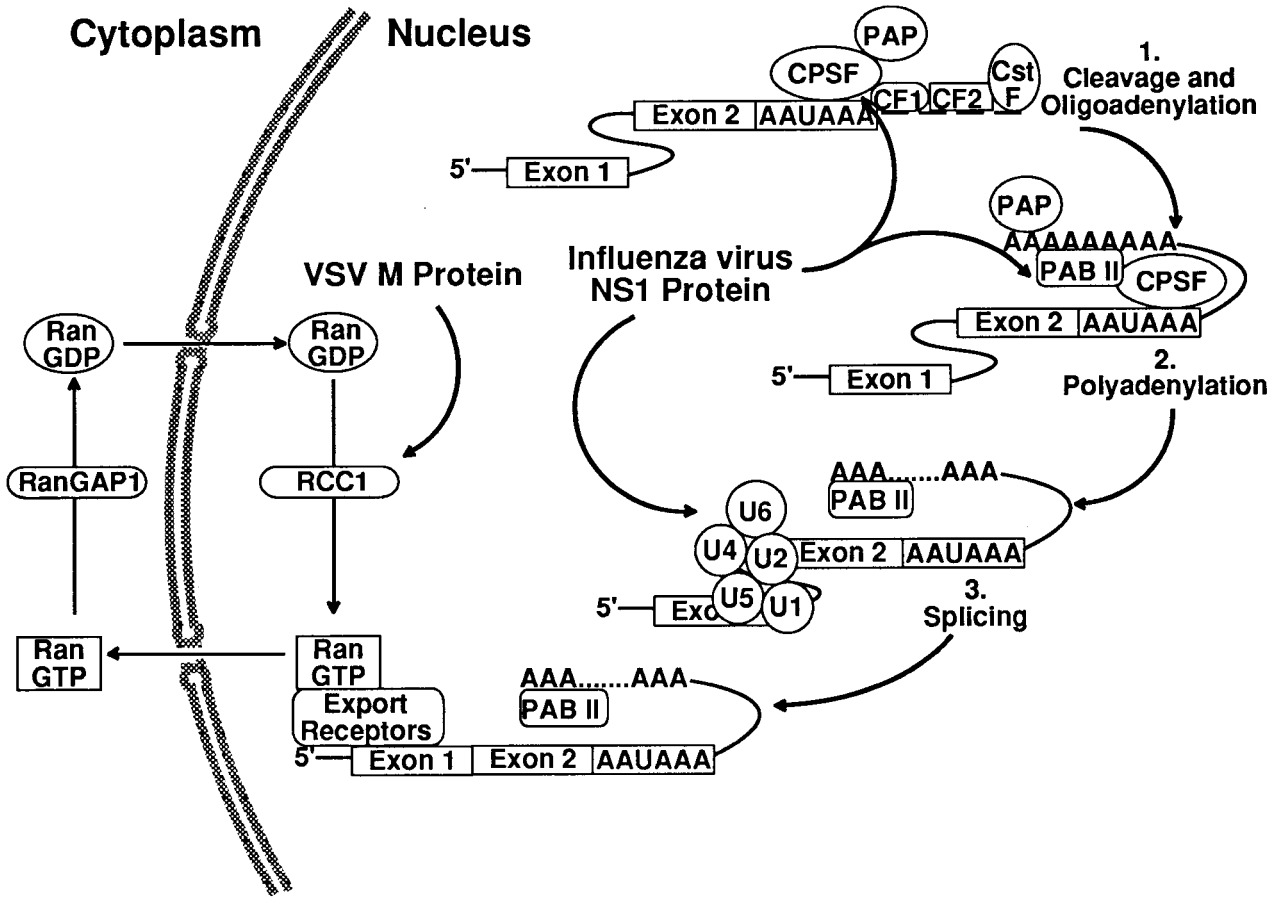

FIG. 2. Processing and transport of cellular mRNAs. Step 1 (clockwise from right): CPSF binds to the AAUAAA sequence of the mRNA precursor; other proteins involved in cleavage and oligoadenylation are cleavage factors 1 and 2 (CF1, CF2), cleavage stimulation factor (CstF), and poly(A) polymerase (PAP). Step 2: Oligonucleotide A at the 3′ end of mRNA precursor is bound by PABII, which stimulates polyadenylation by PAP. Step 3: The mRNA precursor is spliced by U1, U2, U3, U4, U5, and U6 snRNPs. The processed mRNA is transported to the cytoplasm by export receptors that require Ran-GTP. Hydrolysis of GTP by Ran is stimulated by RanGAP1 in the cytoplasm, which causes the release of mRNA. Exchange of the GDP of Ran for GTP is stimulated by RCC1 in the nucleus. The sites of inhibition by influenza virus NS1 protein are indicated. Inhibition by VSV M protein is shown at the RCC1 step because it resembles inhibition by a *ts* mutation in RCC1, but the actual target could be some other step in the Ran-GTP/Ran-GDP cycle.

hibition of processing and assembly of snRNAs occurs within 1 h and thus is probably the earliest effect of VSV on host gene expression (43). Likewise, transcription of cotransfected target genes in mammalian cells that express M protein is profoundly inhibited (2), making it difficult to assay an M protein-induced block in transport. However, there is little if any M protein-induced inhibition of transcription in *Xenopus laevis* oocytes, so that the inhibition of RNA transport and processing by M protein is readily apparent in these cells (60).

The RNA-processing events inhibited by M protein are those that depend on nuclear-cytoplasmic transport. Processing of U1 and U2 snRNAs, which occurs in the cytoplasm, is inhibited by M protein. Processing of U3 snRNA and mRNA, which occurs in the nucleus, is not inhibited, although M protein does inhibit the transport of the processed RNA to the cytoplasm (60). Processing of rRNA requires import of newly made ribosomal proteins into the nucleus. Thus, the inhibition of processing of rRNA probably results from an M protein-induced block in protein import. The only naturally occurring RNAs to escape the inhibitory effects of M protein are tRNAs, although subsequently additional RNA sequences that escape the M protein-induced block in transport have been selected experimentally (53).

The pattern of inhibition by M protein, in which transport into and out of the nucleus of most RNAs and proteins except tRNAs is blocked, is similar to that observed in a cell line with a temperature-sensitive (*ts*) lesion in the Ran-RCC1 system, suggesting that M protein acts by inhibiting one or more components of this system (22). Transport of nearly all proteins and RNA into and out of the nucleus depends on the asymmetric distribution across the nuclear envelope of the two different forms of Ran, a small GTP-binding protein (Fig. 2) (reviewed in reference 65). The GTP-bound form of Ran is maintained in the nucleus by a nuclear guanine nucleotide exchange factor, RCC1, while the GDP-bound form of Ran is maintained in the cytoplasm by a cytoplasmic GTPase-activating protein, RanGAP1. Receptors involved in nuclear export bind their substrates in the presence of Ran-GTP in the nucleus and release them in the presence of Ran-GDP in the cytoplasm. Conversely, receptors involved in nuclear import bind their substrates in the presence of Ran-GDP in the cytoplasm and release them in the presence of Ran-GTP in the nucleus. Thus, M protein-induced interference with the gradient of Ran-GTP/Ran-GDP across the nuclear envelope would inhibit most nuclear-cytoplasmic transport. The relative resistance of tRNA transport appears to be due to a lower requirement for Ran-GTP or else to the existence of a transport pathway that does not depend on Ran (22, 60, 65). The key

questions for the VSV-induced inhibition of nuclear-cytoplasmic transport are how M protein manages to interfere with this system. This could be accomplished by interfering with either step in the generation of the Ran-GTP/Ran-GDP gradient. As with the inhibition of transcription, there are two models for how this might be accomplished, i.e., M protein could directly interfere with one or more of the components of this system or M protein could indirectly activate host inhibitory factors.

INHIBITION OF HOST TRANSLATION

There are many viruses whose mRNAs are translated in infected cells while translation of host mRNAs is inhibited. In fact, this is the aspect of 'host shutoff' that is most commonly assayed experimentally. However, in many cases the inhibition of protein synthesis may be due to the antiviral response of the host while the preferential translation of viral mRNAs is due to adaptation of the virus to growth in the presence of such inhibitory mechanisms. Thus, the first key issue in understanding the inhibition of host translation is to determine whether the inhibition is due to viral or host mechanisms. Regardless of whether the inhibition is of viral or host origin, viruses must have a mechanism to circumvent the inhibition so that viral mRNAs are preferentially translated, since viruses and hosts share the same translational apparatus. The mechanism by which viruses avoid the inhibition of translation is thus the second key issue that must be addressed. In most cases, multiple mechanisms are involved in both the inhibition of translation and the viral escape from the inhibition, so that the relative importance of different mechanisms is usually also an issue. In this section, the current understanding of the mechanism of translation inhibition will be discussed followed by what is known of the viral escape from the inhibition for each of the three virus families under consideration.

The question inevitably arises whether the inhibition of host translation is a secondary effect of virus-induced inhibition of earlier steps in gene expression such as host transcription or RNA processing and transport. For most RNA viruses, the inhibition of translation occurs before the inhibition of host mRNA production has much of an effect on the levels of cytoplasmic mRNAs. In addition, there is usually no stimulation of mRNA turnover, so that the cytoplasm of infected cells contains normal levels of host mRNA. When these RNAs are extracted from infected cells, they can be efficiently translated in vitro in reticulocyte lysates, showing that the mRNAs themselves are not inactivated (70, 82). Instead, the inhibition of translation in infected cells is usually due to inactivation of host translation factors. Thus, the experimental task is to determine which translation factors are inactivated and the mechanism of the inactivation.

Inhibition of Translation by Picornaviruses

The cleavage of translation initiation factor eIF4G in picornavirus-infected cells is a classic example of virus-induced inhibition of host translation (reviewed in reference 107). For most picornaviruses this cleavage is mediated by the viral 2A protease. However, for FMDV, cleavage of eIF4G is mediated by the leader (L) protease encoded at the 5′ end of the viral polyprotein open reading frame (107). eIF4G is one of the subunits of the mRNA-binding eIF4F complex, and it mediates the binding of eIF4F and mRNA to the other elements of the initiation complex (Fig. 3). Cleavage of eIF4G separates the domain responsible for association with the cap-binding subunit eIF4E from the domain responsible for association of other subunits of eIF4F with the initiation complex. Thus,

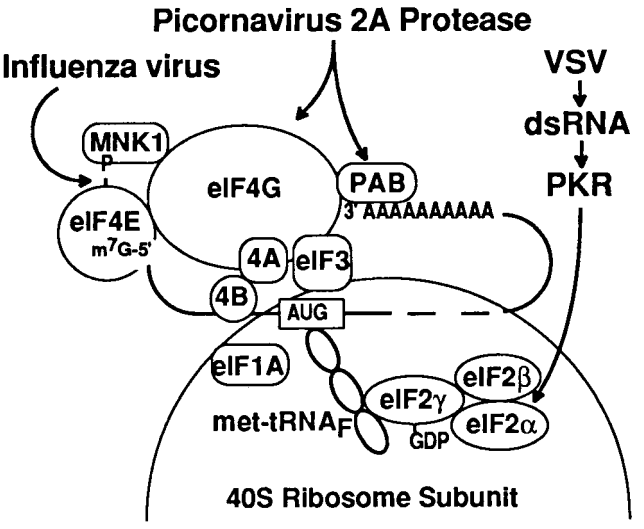

FIG. 3. Formation of the translation initiation complex. mRNA is bound by the eIF4F complex, which consists of eIF4A, eIF4B, eIF4E, and eIF4G. The 5′ end of mRNA is bound by the cap-binding subunit, eIF4E. This requires phosphorylation of eIF4E by the protein kinase MNK1. The 3′ end of mRNA is bound by the cytoplasmic PAB. eIF4G binds eIF4A, eIF4B, eIF4E, PAB, and MNK1 to the other elements of the initiation complex through eIF3. eIF2 binds Met-tRNA$_F$ and GTP and then binds the 40S ribosomal subunit upon hydrolysis of GTP. Proteins which are targets for inhibition by picornavirus 2A proteases, influenza virus, and PKR activated by VSV are indicated.

cleavage of eIF4G inhibits the translation of capped but not uncapped mRNAs. Since RNAs of picornaviruses lack a cap structure, this inhibition does not affect translation of viral mRNA. Much of the debate since the original discovery has been over whether the cleavage is a direct effect of 2A protease or an indirect effect mediated by host proteases and whether there are other mechanisms that contribute to the inhibition of host translation. The idea that cleavage of eIF4G is mediated by host proteases activated by the viral 2A protease came from the observation that cleavage of eIF4G by 2A protease in vitro is very inefficient (16) and that the major proteolytic activity from infected cells that cleaves eIF4G in vitro can be separated from 2A protease biochemically (17, 81). The susceptibility of eIF4G to cleavage by 2A protease (or L protease of FMDV) in vitro is enhanced by interaction with other translation initiation factors (56, 99, 131), although the efficiency of cleavage by 2A protease is still lower than that of cleavage by the host-derived protease (16). Nonetheless, this has led to the proposal that direct cleavage of eIF4G by 2A protease in vivo might be enhanced to the extent that it becomes quantitatively significant in comparison to cleavage by the host-derived protease.

The inhibition of cap-dependent translation has no effect on translation of viral mRNAs because they have an alternative mechanism for translation initiation. Picornavirus mRNAs have a long 5′ noncoding sequence that contains an internal ribosome entry site (IRES), which promotes cap-independent translation initiation. Thus, the key to understanding the selective translation of picornavirus mRNAs is to determine the mechanism by which ribosomes initiate translation at IRES sequences. The weight of evidence supports a model in which cellular proteins bind to secondary-structure elements in the IRES sequence and promote translation initiation by the canonical initiation factors. Several cellular proteins have been identified that fit this model by binding RNAs with viral IRES sequences and enhancing their translation in vitro. These proteins include the cellular autoantigen La (92), a cytoplasmic

form of the nuclear pyrimidine tract-binding protein (59), cytoplasmic poly(rC)-binding proteins 1 and 2 (13, 46), and Unr protein, an RNA-binding protein not previously known to be involved in translational regulation (63). Each of these proteins stimulates the translation of IRES-containing RNAs in vitro, often with additive effects, although IRES sequences from different picornaviruses differ in their responsiveness to some of these proteins (63, 124, 133). How these proteins cause translation initiation factors to bypass the normal requirement for cap-dependent initiation is a key question to be addressed.

The other issue to be considered is the extent to which other mechanisms besides eIF4G cleavage contribute to inhibition of host protein synthesis in picornavirus-infected cells. This question arose from the observation that host protein synthesis was not completely inhibited despite complete cleavage of eIF4G either at early times postinfection or when viral gene expression was partially inhibited (14, 64, 104). However, a novel homologue of eIF4G, called eIF4GII, was recently discovered that has only 46% amino acid identity to the originally identified eIF4G, now called eIF4GI (51). eIF4GII is more resistant to cleavage by picornavirus proteases, and the kinetics of its cleavage more closely parallel those of the virus-induced inhibition of host protein synthesis (52, 123). It is still likely that other mechanisms contribute to the inhibition of protein synthesis in poliovirus-infected cells. For example, eIF4G activity appears to be more important for initiation of translation of newly synthesized mRNAs, whereas mRNAs already engaged in polysomes are less dependent on eIF4G for reinitiation (98). Both picornavirus proteases 2A and 3C cleave the cellular cytoplasmic poly(A)-binding protein (68, 71). This protein participates in translation initiation by coupling elements at the 3′ end of mRNAs with initiation at the 5′ end, referred to as the closed-loop model of translation (Fig. 3). Thus cleavage of the poly(A)-binding protein could contribute to the inhibition of host translation by disrupting this closed loop. Translation initiation factor eIF2 is also inactivated in poliovirus-infected cells as a result of activation of host PKR by viral dsRNA (101). However, inactivation of eIF2 occurs slowly and is more pronounced at late times postinfection. The slow inactivation of eIF2 is due to the virus-induced degradation of PKR, so that effects of PKR activation are not nearly as pronounced in poliovirus-infected cells as in cells infected with other RNA viruses (11).

Inhibition of Translation in Cells Infected with VSV

The inhibition of protein synthesis in cells infected with VSV is an example where the inhibition is due in large part to the antiviral response of the host, particularly the response to dsRNA. Nearly all viruses produce dsRNA as a by-product of virus replication. For many RNA viruses, including VSV, a major feature of the response of host cells to viral dsRNA is the inhibition of protein synthesis by PKR (reviewed in reference 57). While PKR is known primarily as an interferon-inducible kinase, most cells constitutively express substantial amounts of it, and it serves as a major activator of the host response to dsRNA even in the absence of interferon. In the presence of dsRNA, PKR phosphorylates the alpha subunit of the GTP-binding initiation factor eIF2. Phosphorylation of the alpha subunit locks eIF2 in the GDP-bound form, preventing its reutilization for translation initiation (Fig. 3). VSV was an early example of a virus which was shown to induce inhibition of eIF2 by preventing its reutilization, which is a hallmark of PKR activity (20, 34). Furthermore, vaccinia virus proteins that inhibit PKR delay the inhibition of protein synthesis in VSV-infected cells (140). While inhibition of eIF2 appears to be the major mechanism for inhibition of translation in VSV-infected cells, inhibition of the activity of other translation initiation factors may also contribute. Translation in extracts from VSV-infected cells can be stimulated by addition of eIF2 but can also be stimulated by addition of eIF4B and the cap-binding complex eIF4F (34). However, the mechanism of inhibition of these translation initiation factors and whether it is due to viral mechanisms or the host antiviral response has yet to be determined. It is possible that the viral M protein participates in the inhibition of protein synthesis in VSV-infected cells, since a viral mutant containing the M51R M protein mutation shows a delay in the inhibition of protein synthesis as well as host RNA synthesis (35, 122). However, expression of M protein in the absence of other viral gene products does not inhibit translation. Instead, the translation of cotransfected mRNAs is actually enhanced by M protein expression (8). Thus if M protein participates in the virus-induced inhibition of translation, it must do so either together with some other viral component or at higher concentrations in infected cells than in transfected cells. This contrasts with the M protein-induced inhibition of host RNA synthesis, which occurs even in the absence of other viral components and at 1,000-fold-lower M protein concentrations than those found in virus-infected cells (86).

There has been considerable debate for many years about the mechanism of the selective translation of VSV mRNAs compared to host mRNAs in infected cells. An early proposal was that the viral mRNAs simply compete with cellular mRNAs due to their greater abundance (82). In fact, there is a large excess of viral mRNAs that are not associated with polysomes in most VSV-infected cells. These mRNAs are present in an mRNP complex with the viral N protein and may represent a large pool of mRNAs capable of competing with host mRNAs (114). However, viral mRNAs are selectively translated even under conditions in which their relative abundance is reduced by a factor of 10 or more, suggesting that they may have additional mechanisms that contribute to their selective translation (117). It is an attractive hypothesis that viral mRNAs contain sequences that enhance their translation by binding host factors analogous to the IRES of picornavirus RNAs. There is one report indicating that incorporation of viral sequences at the 5′ end of a cellular mRNA can delay the VSV-induced inhibition of its translation. However, this study was flawed by the inclusion of the viral leader RNA sequence as well as the 5′ untranslated region of the viral N mRNA, so that the resulting mRNA did not have an authentic 5′ end of a viral mRNA (7).

Inhibition of Translation in Cells Infected with Influenza Viruses

The inhibition of protein synthesis in cells infected with influenza virus occurs at a step similar to that in cells infected with poliovirus, although a different mechanism is responsible. Inactivation of eIF2 by PKR is not as prominent a feature of influenza virus infection as it is for other RNA viruses like VSV. As in poliovirus-infected cells, the activity of PKR is inhibited, although in influenza virus-infected cells, this is due to activation of a cellular inhibitor of PKR (76). The target of inactivation of protein synthesis in influenza virus-infected cells is the cap-binding initiation factor eIF4E. For eIF4E to bind capped mRNAs, it must be phosphorylated by a cellular kinase, MNK1, which is associated with eIF4G (Fig. 3) (108, 135). In influenza virus-infected cells, eIF4E is at least partially inactivated by reduced phosphorylation (37). Whether this represents lower MNK1 activity or higher phosphatase activity

and whether this is a viral mechanism or a host response to virus infection have not been determined. However, the result would be to reduce the translation of capped cellular mRNAs. Of course, this inhibition would be expected to affect the translation of viral mRNAs as well as those of the host, since the cap and 10 to 13 of the 5′ nucleotides of viral mRNAs are derived from host RNAs by the 'cap-snatching' activity of the viral RNA polymerase. As with poliovirus RNAs, influenza virus mRNAs contain a sequence near the 5′ end that enhances translation in the presence of low levels of translation initiation factors. This sequence includes the 12 5′ nucleotides that are common to mRNAs from all eight genome segments, as well as some of the segment-specific flanking sequences (103). A variety of host proteins can bind to this sequence, and one in particular enhances the translation of viral mRNAs. This protein is the host G-rich sequence factor 1 (GRSF-1), which had been previously identified as an mRNA-binding protein but whose role in translation had not been previously demonstrated (103). Many issues remain to be addressed regarding the way GRSF-1 and perhaps other host proteins enhance the translation of influenza virus mRNAs. However, this clearly extends to influenza virus the principle established with picornaviruses, i.e., that selective translation of viral mRNAs occurs by binding of host factors to *cis*-acting sequences in viral mRNAs that enhance translation under conditions in which the activity of host translation initiation factors is inhibited.

CELL ROUNDING DUE TO VIRUS-INDUCED INHIBITION OF CYTOSKELETAL FUNCTION

During infection with most RNA viruses, cells that are adherent to their substrate undergo pronounced morphological changes leading to cell rounding. A key issue is whether viral gene products directly affect host cytoskeletal components to induce cell rounding or, alternatively, whether cell rounding results from induction of apoptosis in host cells. One of the earliest morphological changes in adherent cells undergoing apoptosis is cell rounding. Thus, the cell rounding that would normally be identified as CPE reflects, in many cases, the induction of apoptosis. This section will consider cell rounding due to disruption of cytoskeletal function by proteins of poliovirus and VSV, and the next section will consider the viral induction of apoptosis.

Inhibition of Cytoskeletal Function by Poliovirus

The cytoskeleton is composed of three major elements: microfilaments, microtubules, and intermediate filaments. Microfilaments are composed of actin subunits, and microtubules are composed of tubulin subunits. These cytoskeletal elements are common to nearly all cell types. Intermediate filaments are composed of cytokeratin subunits that are specific for the state of cellular differentiation. Poliovirus infection probably induces dramatic rearrangements of all three classes of cytoskeletal elements (78). However, the only one for which a mechanism has been demonstrated is the disruption of microtubules. This occurs by cleavage of microtubule-associated protein 4 (MAP4) by the viral 3C protease (67). MAP4 functions in the regulation of microtubule assembly and disassembly, and its effect when bound to microtubules is to stabilize them. Therefore, the effect of MAP4 cleavage would be to promote disassembly of microtubules. While microtubules play a major role in maintaining cell shape, it is likely that disruption of other cytoskeletal elements is necessary to fully achieve the cell rounding seen in virus-infected cells.

While poliovirus-infected cells superficially resemble cells undergoing apoptosis by virtue of being round, there are a number of morphological differences. These include lack of cytoplasmic blebbing and shrinkage, as well as differences in nuclear morphology, in poliovirus-infected cells compared to cells undergoing apoptosis (128). There is also a lack of cleavage of chromosomal DNA into oligomers of nucleosomal length, commonly called DNA laddering, that is characteristic of apoptotic cells (128). However, infection with poliovirus under nonpermissive conditions can give rise to the typical features of apoptosis, indicating that poliovirus has an apoptosis-inducing activity that is normally suppressed in productive infection. The identities of the apoptosis-inducing and the apoptosis-suppressing functions of poliovirus have not been established.

Cell Rounding in VSV-Infected Cells

There are probably two different mechanisms by which VSV induces cell rounding. Like poliovirus, VSV encodes a protein that induces cell rounding by disruption of cytoskeletal function, and like poliovirus, this is the protein responsible for inhibition of host RNA synthesis (3C protease of poliovirus and M protein of VSV). However, VSV can induce cells to undergo apoptosis in response to the production of viral dsRNA, which provides a second mechanism by which VSV can cause cell rounding and cell death. The ability of VSV M protein to induce cell rounding in the absence of other viral components was the first activity of M protein in cytopathogenesis to be described (12). It has been proposed that the cell-rounding activity of M protein results from cytoskeletal changes necessary for virus assembly (146). However, the activity of M protein mutants in cell rounding is clearly correlated with the ability to inhibit host gene expression and is not correlated with the ability to function in virus assembly (85). As in poliovirus-infected cells, cell rounding has been attributed to the disassembly of microtubules both in cells infected with VSV and in cells that express M protein in the absence of other viral components (85, 121). Disruption of intermediate filaments occurs with a similar time course and may also contribute to cell rounding. Actin-containing microfilaments become disorganized but are not disassembled. M protein can inhibit tubulin polymerization in vitro (93). However, the relevance of this effect to microtubule disruption in vivo has been questioned, since a partial proteolysis fragment of M protein resulting from cleavage at position 19 cannot inhibit tubulin polymerization in vitro (93) while a deletion mutant with a similar sequence is as effective as wild-type M protein in causing cell rounding in vivo (85).

INDUCTION OF APOPTOSIS

There are now many examples in which the death of virus-infected cells is due to the active participation of the host cell in the series of biochemical and morphological changes referred to as programmed cell death or apoptosis (115). A key issue is whether apoptosis results from the virus-induced inhibition of host gene expression or, alternatively, is due to the antiviral response of the host. One might expect that viral proteins capable of inhibiting the synthesis, processing, transport, and translation of host RNA would lead to cell death, since inhibition of these processes is inconsistent with cell survival. Indeed, proteins such as VSV M protein and influenza virus NS1 protein are clearly toxic to cells (12, 79, 119). However, the situation in virus-infected cells is not so clear cut. The response of cells to inhibition of gene expression, for example with drugs, varies markedly among different cell types

(reviewed in reference 75). In some cases, inhibition of transcription or translation with drugs such as actinomycin D or cycloheximide rapidly induces an apoptotic response. However, in many cases, cells can survive for a considerable period in the presence of these drugs, and the drugs actually inhibit the apoptotic response to other stimuli. This is because apoptosis often requires the synthesis of new cellular gene products. Thus, viral gene products that inhibit host gene expression might be expected to accelerate apoptosis in some cell types but delay the onset of apoptosis in other cell types. This is a critical issue for viral pathogenesis, since virus-induced apoptosis is widely considered to be a host response to limit virus replication through elimination of virus-infected cells. Despite the importance of this issue, there are few cases where the relationship of the inhibition of host gene expression to the induction of apoptosis has been addressed.

Induction of Apoptosis by Influenza Viruses

Influenza viruses were an early example of viruses shown to cause cell death by apoptosis (reviewed in reference 119). Apoptosis in influenza virus-infected cells is clearly due to the antiviral response of the host. Influenza virus infection induces the surface expression of both Fas (CD95) and Fas ligand (45, 125, 126). Fas is a member of the tumor necrosis factor receptor family, which transduces a proapoptotic signal when bound to Fas ligand. A role for Fas in virus-induced apoptosis is also supported by the activation of the proapoptotic protease caspase 8 but not caspase 9 in influenza virus-infected cells (5). This pattern of caspase activation is typical of receptor-mediated induction of apoptosis such as that induced by Fas. Furthermore, embryonic mouse fibroblasts that lack the adapter protein that connects the Fas receptor to caspase 8 activation (Fas-associated death domain protein [FADD]) are resistant to induction of apoptosis by influenza virus (5). Other ligand-receptor interactions in addition to Fas may also contribute to the induction of apoptosis in influenza virus-infected cells. For example, most cell types secrete a latent form of transforming growth factor β (TGF-β), which can be activated by influenza virus (118). Binding of TGF-β to its receptor can induce apoptosis in many cell types.

A key question is which viral factors are responsible for induction of apoptosis in host cells. For influenza viruses, two factors have been implicated in the induction of apoptosis, viral dsRNA and viral neuraminidase. The weight of evidence supports the idea that the induction of Fas is part of the host response to viral dsRNA. Treatment of cells with synthetic dsRNA induces Fas expression, although induction of apoptosis usually requires additional treatment with an activating antibody against Fas (to substitute for Fas ligand) (125). The induction of Fas expression by either influenza virus or dsRNA is mediated at least in part by PKR. Expression of a dominant negative mutant of PKR prevents the induction of Fas expression and apoptosis, while overexpression of wild-type PKR enhances the induction of apoptosis by both influenza virus and dsRNA (5). This is somewhat puzzling, since activation of PKR is not a prominent feature of influenza virus infection, as noted above when considering the mechanism of inhibition of host protein synthesis. It may be that the small amount of PKR activation that occurs in influenza virus-infected cells is sufficient to induce an apoptotic response or that a certain basal activity of PKR is required for the activity of other factors involved in the response to dsRNA. There may also be other PKR-like kinases that participate in the antiviral response. Cells from transgenic mice lacking the catalytic domain of PKR maintain normal levels of eIF2α phosphorylation, indicating that there are other kinases that can compensate for lack of PKR activity (1). These cells also respond normally to influenza virus infection by undergoing apoptosis (1).

In addition to the host response to dsRNA, induction of apoptosis in influenza virus-infected cells may involve the activity of the viral neuraminidase. Inhibitors of the viral neuraminidase delay the onset of apoptosis when added at early times postinfection (95). Also, viruses with highly active neuraminidase induce apoptosis in host cells more rapidly than do those with less active enzymes (95), although this argument has yet to be fully developed by using single-gene reassortants. The viral neuraminidase has been implicated in the activation of latent TGF-β, in which removal of sialic acid by neuraminidase promotes proteolytic cleavage of the latent form to the active form of TGF-β (118). Thus, influenza viruses may have multiple mechanisms that contribute to the induction of apoptosis in host cells, some associated with the process of RNA replication and others associated with the activity of the viral envelope glycoproteins. This would be analogous to the induction of apoptosis by alphaviruses such as Sindbis virus, in which the ability to induce apoptosis has been linked both to factors associated with RNA replication and to the activities of the envelope glycoproteins (44, 66).

Induction of Apoptosis by VSV

Like influenza viruses, VSV was an early example of a virus shown to cause cell death by apoptosis (74). It is tempting to attribute the induction of apoptosis to the activity of M protein, since it is such a potent inhibitor of host gene expression and also induces cell rounding. However, it is likely that the host response to viral dsRNA, particularly the activation of PKR, plays a major role in the induction of apoptosis in VSV-infected cells, since expression of a dominant negative mutant of PKR delays the onset of apoptosis in VSV-infected cells (5). Although similar in principle, the induction of apoptosis by VSV differs in detail from that of induction by influenza viruses. There appears to be little if any need for Fas or the other elements of the Fas pathway, such as FADD or caspase 8, in apoptosis induced by VSV. Instead, VSV induces the activation of caspase 9 (5). This caspase is usually activated by a mechanism involving the release of cytochrome *c* from mitochondria, which then binds to apoptosis protein-activating factor 1 (Apaf-1) to activate caspase 9. In support of this model, embryonic fibroblasts from transgenic mice that lack Apaf-1 are at least partially resistant to induction of apoptosis by VSV (5). Why VSV and influenza virus induce apoptosis through different pathways is a very interesting question. The answer may be related to the influence of different viral proteins on the apoptotic pathways. The fact that PKR activation is largely inhibited in cells infected with influenza virus but not VSV may play a role. Also, the influence of the viral proteins responsible for inhibiting host gene expression, namely, NS1 protein and M protein, must be evaluated to determine whether they play a role in promoting or delaying the host apoptotic response.

VIRAL TROPISM AND PATHOGENESIS IN VIVO AND IN VITRO

Role of the Host Antiviral Response in Tissue Tropism

There are well-characterized examples of viruses whose tropism for particular tissues is dictated by receptor specificity. These include, for example, the tropism of human immunodeficiency virus for T cells and macrophages containing the CD4 receptor and the tropism of Epstein-Barr virus for B cells

containing the CD21 receptor (reviewed in reference 62). However, the specificity and tissue distribution of receptors cannot account for the tropism of most RNA viruses (62). For example, influenza viruses use as receptors sialic acid residues attached to glycoproteins and glycolipids that are ubiquitously expressed throughout most tissues, yet influenza virus infection is confined exclusively to the respiratory tract in humans and in most animal models of human disease. Likewise, VSV shows little if any receptor specificity, using a variety of cell surface polycationic or polyanionic molecules for virus attachment, yet displays a profound neurotropism in animals.

For these viruses, the major determinant of viral tropism is the ability of cells in different tissues to mount an antiviral defense in response to alpha/beta interferons. This was shown by analyzing the tissue distribution of virus replication in transgenic mice defective for one or more components of the alpha/beta interferon signal transduction pathway. In normal mice, replication of most influenza viruses is confined to the respiratory tract, as it is in human influenza virus infections. However, the pattern of virus replication is quite different in mice either lacking one of the subunits of the alpha/beta interferon receptor or lacking the STAT1 transcription factor subunit that mediates the response to alpha/beta interferon (47). In these mice, influenza viruses cause a systemic infection characterized by virus replication in many organs. Similarly, in normal mice, VSV is confined almost exclusively to neural tissue, particularly the central nervous system. However, in mice lacking one of the subunits of the alpha/beta interferon receptor, VSV replicates in nearly every organ examined and actually grows to the lowest titers in the central nervous system (96). The principle illustrated by these experiments is that these viruses display a tropism not necessarily for the tissue that is most capable of supporting virus replication but instead for the tissue that is least able to mount an antiviral response to alpha/beta interferons. This does not mean that host factors that enhance virus replication do not play a role in tissue tropism, nor that other host defense mechanisms are not involved. However, these results clearly focus our attention on the ability of different tissues to produce and respond to alpha/beta interferon as a major determinant in viral tropism and pathogenesis.

The ability of host cells to secrete alpha/beta interferons is usually viewed as a race between the ability of the host to respond to virus infection and the ability of the virus to inhibit the host response. For most viruses, the major inducer of alpha/beta interferon gene expression is viral dsRNA (138). Because of the potency of dsRNA at inducing an antiviral response, most replicative intermediates of RNA viruses occur in association with viral proteins that sequester double-stranded regions of viral RNA. Thus, little if any free dsRNA is produced as part of the virus replicative cycle. Instead, dsRNA is a minor by-product of virus replication, either due to errors in the formation of normal viral ribonucleoproteins or due to cellular catabolism of viral ribonucleoproteins that releases free positive- and negative-sense RNA that can form a dsRNA hybrid. Since the origins of viral dsRNA are somewhat obscure, there is much to learn about the viral and host factors that determine how much dsRNA is produced in different cell types. Nonetheless, this is probably a critical element in the race between virus and host. The contribution of viral factors that inhibit the host interferon response is perhaps better understood than is the origin of the dsRNA that induces the interferon response, although there many questions remain to be addressed in this area as well.

Inhibition of the Host Antiviral Response by NS1 Protein

The importance of inhibition of the host response to dsRNA is illustrated by the phenotype of influenza viruses with mutations that inactivate NS1 protein. A virus completely lacking the NS1 gene replicates reasonably well in cells such as Vero cells, which are defective in alpha/beta interferon expression (48). Thus, NS1 protein is not essential for the replication of influenza virus. However, replication of virus lacking the NS1 gene is severely inhibited in cells capable of secreting and responding to alpha/beta interferon, such as MDCK cells. This result implies that the primary function of NS1 protein is to inhibit the antiviral response of the host. Even in Vero cells, there is about a 10-fold difference in virus yield between wild-type influenza virus and virus lacking the NS1 gene (48). This is probably due to the ability of NS1 protein to inhibit antiviral responses that are independent of alpha/beta interferon.

NS1 protein has two different types of activities that would be expected to inhibit the host antiviral response. One activity is the ability to bind dsRNA and sequester it from interaction with host proteins. This activity requires only the RNA-binding domain of NS1 protein (84, 110). The other type of activity is the ability to inhibit the processing and transport of host mRNAs. These activities would be expected to inhibit the production of mRNA for interferons as well as mRNAs for interferon-inducible proteins. The inhibition of mRNA processing and transport requires both the RNA-binding and the activation domains of NS1 protein (90, 109). Both the ability to bind dsRNA and the ability to inhibit mRNA processing and transport appear to be important for inhibition of the host antiviral response, since viruses with truncation mutations in NS1 protein replicate progressively less well as more of the activation domain is deleted (36).

These results emphasize the importance of both the dsRNA-binding activity of NS1 protein and its ability to inhibit host gene expression for influenza virus replication in cells capable of producing alpha/beta interferons. In cells in culture, alpha/beta interferons act in an autocrine fashion, i.e., cells both produce and respond to interferons. It is expected that NS1 protein would inhibit both the induction phase and the response phase, since both phases depend on the presence of viral dsRNA and both depend on the synthesis of new host gene products. However, the different activities of NS1 protein may assume different relative levels of importance for the induction and response phases. Also, the various activities of NS1 protein may assume different levels of importance in different cell types. This may be particularly true for influenza virus infection in intact animals, where the alpha/beta interferon response may be more paracrine in nature; i.e., the cells that produce interferons are different from those primarily responsible for producing virus. For example, influenza virus-infected macrophages, although less permissive for virus replication than respiratory epithelial cells, may be the primary producers of alpha/beta interferons (138). Respiratory epithelial cells, which are the primary sites of replication of influenza virus, would therefore be the primary responders to alpha/beta interferons.

Inhibition of the Host Antiviral Response by M Protein

Similar arguments for the role of influenza virus NS1 protein in inhibition of the host antiviral response can be made for the VSV M protein. Unlike NS1 protein, however, M protein is essential for virus replication because of its role in virus assembly. Also, VSV is not known to encode a protein capable of sequestering dsRNA, analogous to the dsRNA-binding activity of NS1 protein. Therefore, the only known activity of M pro-

tein that could play a role in inhibition of the host antiviral response is its ability to inhibit host gene expression. Perhaps for this reason, VSV is usually much more sensitive to the action of interferons than are influenza viruses.

VSV mutants containing the M51R M protein mutation, which reduces the ability of M protein to inhibit host gene expression, replicate as well as wild-type VSV in cells such as BHK cells, which do not respond to alpha/beta interferons (3, 28, 122). However, in cells capable of producing and responding to alpha/beta interferons, such as chicken embryo cells, the replication of these viruses is severely compromised, particularly at elevated temperatures, so that one of these mutants (*ts*O82) was originally identified as a ts mutant (40). Infection with viruses containing M gene mutations, such as *ts*O82 virus, results in production of much more alpha/beta interferon than does infection with the wild-type viruses from which they were derived (15, 89), supporting a role for M protein in suppressing the induction phase of the alpha/beta interferon response. There is one example of a VSV mutant in which infected cells produce more alpha/beta interferon than do cells infected with the wild-type strain from which this mutant was derived, yet this mutant does not contain a mutation in its M protein (89). It has been argued that this mutant rules out a role for M protein in the inhibition of interferon production. However, a more likely explanation is that this virus contains a mutation that makes it a more efficient inducer of alpha/beta interferons, for example, by increasing the production of dsRNA. Wild-type field isolates as well as laboratory strains of VSV vary over nearly 3 orders of magnitude in their ability to induce alpha/beta interferons in chicken embryo cells (88). This variability probably reflects the interplay of many different potential genetic combinations that affect both the interferon-inducing activities of VSV, such as production of dsRNA, and the interferon-inhibiting activities, such as those of M protein.

CONCLUSION

Viral cytopathogenesis is clearly a result of the balance between the antiviral response of the host and the viral mechanisms which inhibit that response. The graphs in Fig. 4 show models of how this balance occurs as a function of time postinfection in four different types of virus-host interaction. For simplicity, only two viral factors are shown. The production of dsRNA is meant to be the prototype for a viral gene product that provokes an antiviral response in host cells. This might also include, for example, the activities of viral envelope glycoproteins to which the host responds. The viral proteins that inhibit host gene expression would include such prototypes as poliovirus 3C and 2A proteases, influenza virus NS1 protein, and VSV M protein. Three different host responses are shown. The inhibition of total mRNA synthesis is meant to represent the result of all of the viral inhibitory mechanisms, including inhibition of transcription, processing, and transport of mRNA. The synthesis of alpha/beta interferon mRNA is shown as a balance between stimulation by dsRNA and inhibition that parallels the overall inhibition of host mRNA synthesis. Similar patterns would be expected for mRNAs encoding any proteins involved in the antiviral response that are induced after infection, such as interferon-inducible proteins and antiviral proteins induced in cells that do not respond to alpha/beta interferons. Finally, the progression of apoptosis is shown to represent a process that is promoted both by viral inhibitory proteins and by the host response. Similar considerations would govern, for example, the inhibition of host protein synthesis. For the sake of argument, the apoptotic response to viral proteins that inhibit host gene expression is assumed to occur more slowly than the apoptotic response to dsRNA and interferon, and the viral proteins are assumed to initially inhibit the response to dsRNA.

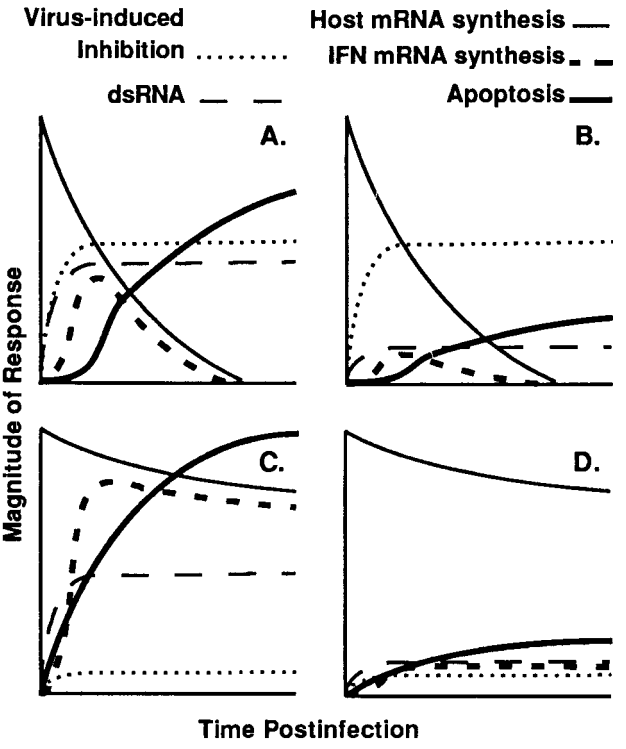

FIG. 4. Models for the balance between virus-induced inhibition of host gene expression and the host antiviral response. The virus-induced inhibition of host gene expression includes the effects of such proteins as poliovirus 3C and 2A proteases, influenza virus NS1 protein, and VSV M protein. The production of dsRNA is meant to be the prototype for a viral gene product that provokes an antiviral response in host cells. Three different host responses are shown. The inhibition of total host mRNA synthesis represents the result of all of the viral inhibitory mechanisms, including inhibition of transcription, processing, and transport of mRNA. The synthesis of alpha/beta interferon (IFN) mRNA is shown as a balance between stimulation by dsRNA and inhibition that parallels the overall inhibition of host mRNA synthesis. The progression of apoptosis is shown as a process that is promoted both by viral inhibitory proteins and by the host response. For the sake of argument, the apoptotic response to virus-induced inhibition of host gene expression is assumed to occur more slowly than the apoptotic response to dsRNA and interferon and the viral proteins are assumed to initially inhibit the response to dsRNA. (A) Typical pattern of virus-host interaction for RNA viruses. (B) Pattern of virus-host interaction for cells that are less capable of responding to viral dsRNA, for viruses that produce less dsRNA, or for viruses that are more effective in the inhibition of host gene expression. (C) Pattern of virus-host interaction for viral mutants that are defective in the inhibition of host gene expression or for cells that are hyperresponsive to dsRNA. (D) Pattern of virus-host interaction for noncytopathic viruses.

Figure 4A shows a typical virus-host interaction for wild-type influenza virus or VSV in cell culture. Cells initially respond to the presence of viral dsRNA by producing interferon mRNA, but its synthesis is limited by the action of viral proteins that inhibit host gene expression. Viral dsRNA also induces an apoptotic response, although its onset is delayed by the virus-induced inhibition of host gene expression. Figure 4B shows a virus-host interaction that would be more typical of poliovirus infection, in which the host response to dsRNA does not play as prominent a role. This could be due to more effective inhibition of host gene expression by poliovirus than by influenza virus or VSV or to less of a propensity for the viral polymerase to generate dsRNA. This pattern would also be seen in cells

that are less able to respond to dsRNA, such as those that express a dominant negative mutant of PKR (5). In this case, the actual amount of dsRNA may not be different but the magnitude of the host response would be lower. In such cells, very little interferon would be produced and the onset of apoptosis would be delayed. This combination would be expected to be very favorable for virus replication. Because the onset of apoptosis is delayed, such a pattern of virus-host interaction in cell cultures might not be interpreted as very cytopathic for many viruses, based solely on the induction of morphological changes associated with apoptosis. However, this is probably the pattern most closely related to viral pathogenesis in intact animals, since, as pointed out in the previous section, the tissues most strongly affected by viruses are often those that are least able to respond.

Figure 4C shows a virus-host interaction in which there is relatively little inhibition of host gene expression relative to the host response to dsRNA. This would be the case for influenza virus mutants defective in expression of NS1 protein or VSV mutants containing M gene mutations that reduce its ability to inhibit host gene expression (3, 36, 48, 122). This pattern could also result from viral mutants that produce more than the normal amounts of dsRNA or from host cells that are hyperresponsive to dsRNA, such as those that overexpress PKR (5). In these cases, cells produce the largest amounts of interferon and undergo the most rapid apoptosis. This pattern in virus-infected cell cultures would be interpreted as the most cytopathic based on morphological criteria, due to the rapid onset of apoptosis. However, this pattern would be the least favorable for virus replication.

Finally, Fig. 4D shows a pattern of virus-host interaction rarely exhibited by the viruses considered in this review, since it has few of the features associated with viral cytopathogenesis. However, many RNA viruses have little if any harmful effect on cells in culture. These viruses do not appear to have viral proteins that are potent inhibitors of host gene expression, and virus-induced apoptosis occurs slowly if at all. Such viruses readily establish persistent infections in which the infected cells survive and continue to produce virus in the absence of a notable antiviral response. Examples include viruses related to ones considered here. For example, rabies virus, although a member of the rhabdovirus family like VSV, shows very little CPE in cell culture (19), and its M protein has little if any activity in inhibition of host gene expression in cotransfection experiments (B. McKnight, M. O. McKenzie, and D. S. Lyles, unpublished data). Likewise, many members of the paramyxovirus family do not inhibit host RNA synthesis. These viruses may have more subtle ways to inhibit the host antiviral response than by inhibiting overall host mRNA synthesis. For example, the simian virus 5 nonstructural protein V and the Sendai virus nonstructural protein C prevent the establishment of an antiviral state in response to alpha/beta interferon (32, 33, 49, 50). In simian virus 5, the V protein induces the degradation of the transcription factor STAT1 (33), although this does not appear to be the case for the Sendai virus C protein (49). Determination of the mechanisms by which these effects are accomplished will be very interesting because of their importance for viral pathogenesis.

The graphs represented in Fig. 4 are meant to be models of virus-host interaction. There are many questions to be addressed about the mechanisms involved in the virus-induced inhibition of host gene expression and the host antiviral response, as well as the relationships among the different mechanisms in terms of their relative importance for any particular virus-host combination. The focus of this review has been on host responses and their inhibition by viruses at the level of individual infected cells. However, similar principles will apply to virus-host interactions in intact organisms. The curves in Fig. 4 that represent dsRNA will be joined by those for other viral components that provoke an antiviral response, such as inducers of other cytokines besides alpha/beta interferons involved in innate immunity, as well as viral antigens capable of eliciting acquired immunity. Likewise, viral mechanisms that inhibit host responses in intact animals may involve more than the inhibition of host gene expression. Nonetheless, the patterns in which these factors are balanced in intact animals are likely to be very similar to the patterns observed for viral cytopathogenesis.

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