REVIEW

RNA Viruses as Vectors for the Expression of Heterologous Proteins

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Abstract

RNA viruses comprise a wide variety of infectious agents, some of which are the cause of disease in humans, animals, and plants. Recombinant DNA technology is now making it feasible to modify these genomes and engineer them to express heterologous proteins. Several different schemes are being employed that depend on the genome organization of the virus and on the strategy of replication of the particular virus. Several different examples are illustrated and potential uses as well as possible problems are discussed. In the future reverse genetics may convert some of these viruses from agents of disease to agents of cure.

Index Entries: RNA viruses; alphaviruses; Sindbis virus; Semliki Forest virus; picornaviruses; poliovirus; influenza virus; RNA virus expression vectors.

1. Introduction

A role for viruses as vectors to introduce heterologous genes into cells can be traced back to the use of bacteriophage as transducing agents. It was not until the development of recombinant DNA technology, however, that the use of viruses to introduce a wide variety of genes into bacterial, animal, and plant cells became feasible. Most of the viruses that are currently being used as vectors contain DNA genomes or are retroviruses that contain an RNA genome that is replicated through a DNA intermediate. There is increasing evidence that RNA viruses can also be used as expression vectors. But why RNA viruses? One reason is that some of these viruses produce very high levels of their proteins in the cells they infect and as vectors they could be adapted for the large scale production of a protein. In addition, there are several RNA viruses that are associated with human diseases, but which have been attenuated and developed as vaccines. Perhaps these viruses can be engineered to express other proteins as immunogens. This would allow vaccination against some agents that have so far resisted more conventional methods of attenuation. One issue that has been raised in the use of DNA viruses and retroviruses as vectors is that their ability to integrate their genomes into host chromosomes could lead to genetic damage. In contrast, infection of a cell by one of the RNA viruses that replicates entirely in the cytoplasm and does not require any nuclear functions should not lead to any permanent genetic alterations in the host. Finally, RNA viruses have provided useful tools in cell biology and their ability to express specific cellular proteins would permit their use in studies involving posttranslational modifications and localization of specific proteins in a variety of different types of cells.

There are a number of different schemes that are being devised for developing RNA viruses as vectors. In most cases the virus retains its ability to be an infectious agent. One approach has been to superimpose the heterologous gene onto a complete infectious viral genome. A modification of this is to introduce the heterologous gene into a defective viral genome—one that contains the cis-acting sequences essential for the RNA

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to be replicated and encapsidated into viral particles, but that lacks some or all of the genes that code for the proteins required for these functions. This type of defective genome will be amplified and will express its coding sequences if it is complemented by a virus able to provide the missing functions. Both of these examples lead to the production of an infectious virus vector capable of spreading from cell to cell or from organism to organism. Some virus genomes are being modified to retain the replication, transcription, and translation abilities of the original genome, but not its ability to be packaged into particles. These vectors would not spread as infectious agents. Another scheme involves the insertion of heterologous sequences within a gene that codes for a viral structural protein in such a way that the virus retains viability and the heterologous peptide becomes a part of the viral protein. In this case the viral protein must be able to tolerate such an alteration and still maintain its biological function. Before discussing the promises and potential problems of RNA viruses as vectors, I describe some aspects of the replication of these viruses that are relevant to understanding some of the strategies that are being employed.

The genomes of RNA viruses are either of positive (+) or negative (–) polarity *(1)*. Positive strand RNA genomes are able to function as mRNAs and RNA isolated from a virus with a (+) strand genome is infectious. When a (+) strand RNA genome enters the cell cytoplasm it is translated into enzymes required for transcription and replication of the viral RNA. Negative or (–) strand RNA genomes are the complements of mRNAs. RNA viruses that contain a negative strand genome also contain within the ribonucleoprotein of the virus particle enzymes that are able to transcribe the (–) strand into (+) strands. In an infected cell, these enzymes are able to transcribe the genomic RNA into mRNAs that are then translated into the proteins required for the complete replication cycle and packaging of the virus.

Differences in polarity have played an important part in the progress that has been made in analyzing RNA genomes by reverse genetics—the term used to describe the conversion of an RNA genome into a complementary DNA (cDNA) by an RNA-dependent DNA polymerase (reverse transcriptase). The viral genomic cDNAs are placed directly downstream of a promoter for a bacterial or bacteriophage DNA-dependent RNA polymerase within the general context of a bacterial plasmid. This cloning step permits the viral genome to be subjected to a variety of modifications such as deletions, additions and mutagenesis using recombinant DNA technologies. Usually, transcription of the cDNA back into RNA is then carried out in the test tube after which the RNA is introduced directly into cells. This procedure has worked well for (+) strand RNA viruses because, when introduced into cells, these genomes can initiate an infectious cycle. Strategies for converting cloned cDNAs of (–) strand RNA genomes into infectious RNAs are more complicated because no translation would occur in the absence of the proteins required for transcribing them into mRNAs *(see later)*.

2. Positive Strand RNA Viruses as Vectors for the Expression of Heterologous Genes

A wide variety of animal and plant RNA viruses have (+) strand RNA genomes. At first glance the translational strategies of these genomes might be considered diverse, but there are basically two themes with variations. The first theme is the initiation of translation at a single open reading frame with all of the individual viral proteins generated by co- and posttranslational proteolytic cleavages. The second theme is for some of the viral proteins to be translated from the genomic-length RNA and some to be translated from subgenomic mRNAs. Picornaviruses and togaviruses are two animal virus families whose members are being adapted for use as vectors. They illustrate how the two themes of translation have dictated the strategies for this adaptation.

2.1. Picornaviruses

Historically, the most important member of the picornavirus family is poliovirus, but this family contains a large number of other human and animal pathogens. There are four genera and in addi-

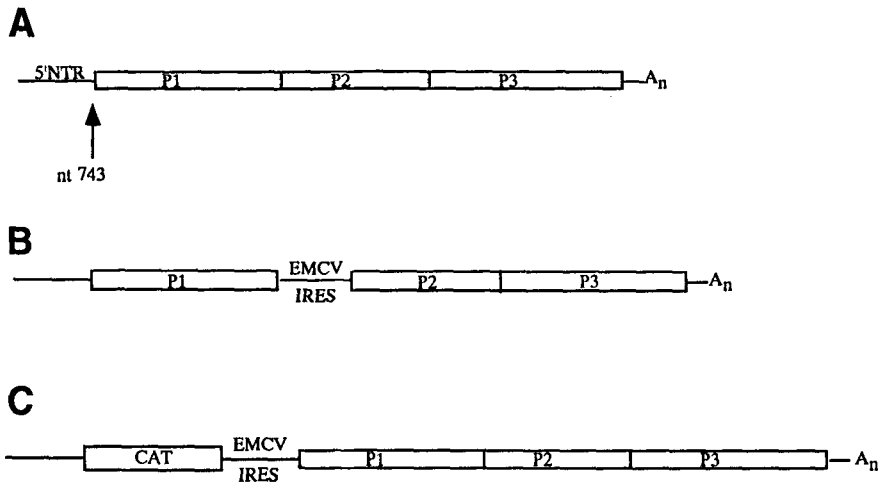

Fig. 1. Organization of the poliovirus genome and of dicistronic variants. **(A)** Organization of the genome of poliovirus. The line at the 5' and 3' ends represents the nontranslated regions (NTR) of the genome. The IRES for the poliovirus genome is in the 5' NTR. The open box represents the single open reading frame that begins at nt 743. P1 codes for the viral structural proteins. P2 includes the coding region for the viral proteinases and 3' end of P3 is the coding region for the viral polymerase. **(B)** The poliovirus genome as a dicistronic mRNA. In this engineered construct, the P1 segment of the genome is separated from P2 and P3 by the IRES of EMCV *(8)*. The RNA is transcribed from cDNA under the control of the T7 promoter. **(C)** The poliovirus genome as a genetic hybrid. The 5' NTR of the poliovirus genome is upstream of the gene for CAT. The EMCV IRES is downstream of the CAT gene and controls the expression of the poliovirus genes *(9)*. The RNA is transcribed from cDNA under the control of the T7 promoter.

tion to the enterovirus genus, which includes poliovirus and human hepatitis A virus, there is the rhinovirus genus, which consists of over 100 serotypes of the virus that is the major cause of the common cold *(2)*. The detailed information now available about the structure and replication strategy of these viruses (reviewed in ref. *3*) has been an essential part of the development of the genomes as vectors.

Poliovirus genomic RNA contains approximately 7500 nucleotides (nt). It has a polyA tract at the 3' terminus, but the 5' end is distinct from most eucaryotic mRNAs, which have their 5' terminus capped with a m^7Gppp. Instead, the genomic RNA (of all picornavirus genomic RNAs) contains a protein (Vpg) at the 5' terminus, but the viral RNA on polyribosomes has the sequence pUpUpA at the 5' terminus (reviewed in ref. *3*). The schematic drawing shown in Fig. 1A illustrates two features of the poliovirus genome: the structural protein genes are located at the 5' terminus followed by the nonstructural protein genes (*see* Fig. 2 for the reverse) and the AUG that initiates translation is located over 700 nt from the 5' terminus. The identification of an AUG at that position as the initiating codon and the presence of AUGs in an optimal context for translation further upstream had suggested that ribosomes might not follow a scanning mechanism in initiating translation of the poliovirus mRNA (reviewed in ref. *4*). For mRNAs that are capped, the 40S ribosomal subunit binds at the 5' terminus and scans the RNA until reaching an initiation codon *(5)*. Depending on the context of the first AUG, translation initiation may occur only at that AUG or that AUG may be partially bypassed in favor of a downstream AUG in a more favorable context. The initiation of translation of picornavirus RNAs, however, occurs by a different mechanism at an internal ribosomal entry site (IRES). Internal initiation was first demonstrated with an RNA containing two reporter genes in tandem; the first downstream of a 5' terminal cap and the second downstream of an

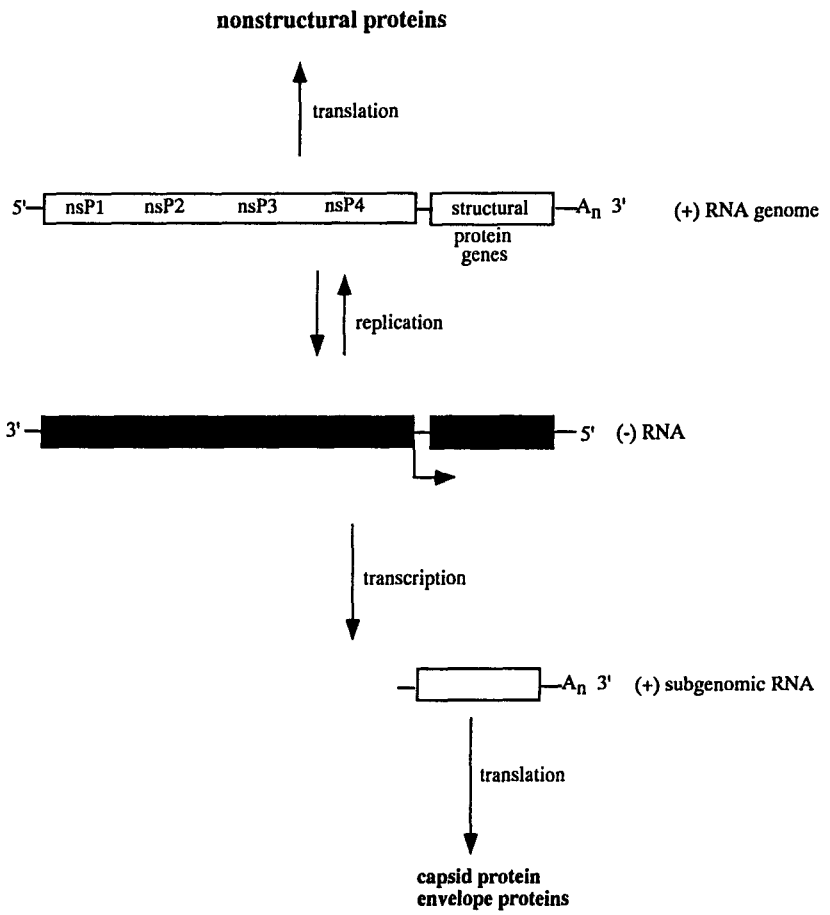

Fig. 2. The scheme for the replication of the alphavirus genome. In the infected cell the 5' 2/3s of the (+) strand RNA genome is translated into the nonstructural proteins that are required for replication and transcription of the RNA. Translation is initiated at a single site; the nonstructural proteins are cleaved into four polypeptides (nsP1–4). The (–) strand RNA is the template for the (+) strand genome and the (+) strand subgenome; the latter is translated into the viral structural proteins. The horizontal arrow (shown in the (–) strand) represents the promoter for the subgenomic RNA.

IRES. Translation of the second reporter gene was not dependent on translation of the upstream sequences *(6,7)*. Subsequently, a poliovirus RNA was engineered in which the coding sequences of the genome were interrupted by placing an IRES from another picornavirus, encephalomyocarditis virus (EMCV) between the sequences coding for structural proteins and nonstructural proteins (Fig. 1B). This dicistronic RNA was infectious—and gave rise to new infectious particles *(8)*.

This construction served as a model for engineering poliovirus to express a foreign gene. The cDNA of the poliovirus nontranslated region including the poliovirus IRES was positioned directly downstream of the promoter for the T7 DNA dependent RNA polymerase and upstream of the chloramphenicol acetyltransferase (CAT) gene followed by the EMCV IRES followed by the complete poliovirus genome (Fig. 1C). RNA transcribed from this cDNA produces functional poliovirus as well as the CAT protein *(9)*. The virus genome was 17% longer than that of the original poliovirus and the larger genome was not stable with passaging. Although CAT activity could be measured in cells infected with virus that had been passaged five times, the activity decreased and deleted genomic RNAs became evident. Furthermore, when a heterologous gene

larger than CAT was inserted into the genome, packaged virions were not obtained. Thus the picornavirion structure appears to have size constraints and only limited amounts of foreign sequences are tolerated.

An earlier approach to engineering picornaviruses to carry foreign sequences was based on the three-dimensional structure of the virion particle. VP1 is the major virus structural protein in the virion and X-ray structural analysis of the particle indicated a region that is in a relatively flexible loop. Using recombinant DNA technology it was possible to insert sequences coding for small peptides into this region of the genome and to generate infectious virions containing amino acids different from or in addition to the original. The viruses formed appear to be stable and initial studies have indicated that these viruses can be used as immunogens and that infected animals can mount an immune response against the inserted peptide (reviewed in ref. *10*). This strategy has also been demonstrated with rhinovirus *(11)*. One of the attractions of using a virus such as poliovirus to deliver other immunogens into human populations is the long experience that exists with vaccinations of attenuated strains of this virus. Of course, all of the disadvantages accompanying the use of attenuated polioviruses will exist as well as the additional one that many people have already been vaccinated with these viruses and would not be sensitive to a new infection with a chimeric virus.

2.2. Togaviruses

The togavirus family consists of two genera—the alphaviruses and the rubiviruses *(12)*. Rubella virus, the cause of German measles, is the only member of the rubivirus genus, but the alphavirus genus includes the eastern, western, and Venezuelan encephalitis viruses, which are all serious human pathogens. It also includes both Sindbis and Semliki Forest viruses. The latter two are not usually associated with human diseases, instead they are best known for providing valuable tools for basic studies in cell and molecular biology. They are now being developed as vectors for the expression of heterologous proteins *(13,14)*. The alphavirus genomic RNA contains approximately 1.2×10^4 nt and has two open reading frames (ORFs) separated by a cluster of termination codons. Only the first two-thirds of the genome is translated from the genomic RNA owing to these termination codons (Fig. 2). This region codes for the proteins that are required for transcription and replication of the RNA (reviewed in refs. *12,15*). Translation of these nonstructural proteins is followed by the synthesis of genomic length complementary RNA [(–) strand], which then serves as a template for the synthesis of genomic RNA and a subgenomic RNA that is identical in sequence with the 3′ one-third of the genome. This subgenomic RNA contains the second ORF that codes for the viral structural proteins—a capsid protein required for the formation of the viral ribonucleoprotein (nucleocapsid) and two viral glycoproteins that are essential for the production of extracellular, infectious virions. Both the nonstructural and structural proteins are translated from single initiation sites and are proteolytically processed by co- and posttranslational cleavages. For the nonstructural proteins the proteolytic cleavages are carried out by one of the proteins that is embedded in the polyprotein. For the structural proteins, the capsid protein that is the protein at the amino terminus of the polyprotein is an autoprotease. The cleavages of the viral membrane proteins are carried out by host cell enzymes.

Two types of RNA expression vectors derived from the infectious alphavirus genome have been described; both take advantage of the high level of subgenomic RNA which accumulates in infected cells (reviewed in refs. *13,14*). One vector contains two subgenomic RNA promoters leading to synthesis of two subgenomic mRNAs; one controlling expression of the heterologous product and the other synthesis of the Sindbis virus structural proteins (Fig. 3A). This vector is self-replicating, produces infectious virus particles, and can spread from cell-to-cell in a manner similar to the parental virus. Such vectors have proven to be of value for studies in immunology *(16)* and cell biology *(17)*. In the second type of vector, heterologous sequences replace the structural protein coding genes (*18,19;* Fig. 3B). Such

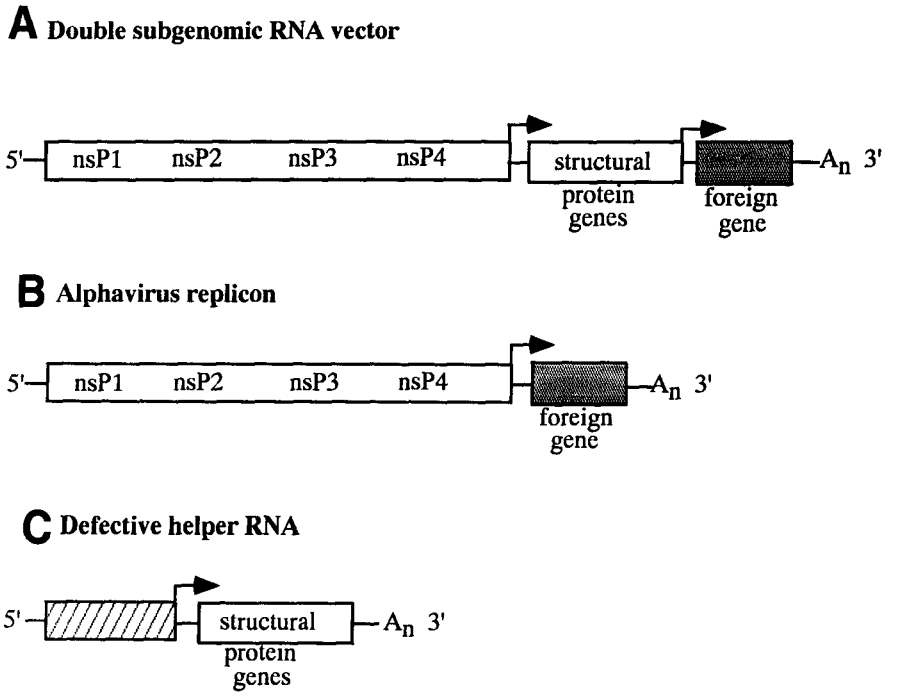

Fig. 3. Organization of alphavirus genomes. The horizontal arrows represent the promoters for transcription of the subgenomic RNA. **(A)** The double subgenomic RNA vector. In this illustration the foreign gene is placed downstream of the structural protein genes; the opposite orientation can also be used *(13,16)*. **(B)** The replicon lacking the viral structural protein genes which are replaced by heterologous sequences *(13,14,18–20)*. **(C)** A defective helper RNA. The defective RNAs contain large deletions in the nonstructural protein genes. The packaging signal for the Sindbis virus defective RNAs spans nt 745–1225 and is deleted in the defective helper RNAs that are packaged very poorly *(22)*. When the replicon and helper RNAs are cotransfected into cells each complements the other—the replicon provides the enzymes for replication and transcription of both viral RNAs, the defective helper RNA provides the proteins for packaging of the replicon.

recombinant RNA genomes, referred to as replicons, can be efficiently introduced into some cell types as naked RNA by electroporation and lead to high levels of heterologous gene expression. They can be packaged by the use of defective helper RNAs also derived from the alphavirus genome *(18,20)*. These helper RNAs contain the cis-acting sequences at the 5' and 3' termini of the genome that are essential for replication and they contain the genes for the structural proteins directly downstream of the sequences that encompass the promoter for transcription of the subgenomic RNA (Fig. 3C). They lack a major portion of the nonstructural protein genes as well as the cis-acting sequences defined as the encapsidation signal. In initial studies with Sindbis virus, the helper RNA contained sequences within the nonstructural protein genes that have been identified as an encapsidation signal and also appear to enhance RNA replication. In that case the helper RNA was also packaged and there appeared to be a high level of co-packaging of the RNAs leading to the formation of a virus with a bipartite genome that gave rise to plaques and could be passaged from cell to cell *(21)*. Helper RNAs that lack these sequences have been derived from both Sindbis virus and Semliki Forest virus genomes *(18,22)*. They replicate poorly and are not packaged into particles or are packaged to very low levels. Under conditions in which the helper RNA is not packaged the alphavirus replicon "commits suicide." As a virion particle it can enter and infect cells very efficiently. The RNA is translated, new genomic RNA is rep-

licated, subgenomic RNA is transcribed and the coding sequences inserted into the subgenomic RNA are translated. In the absence of the synthesis of the structural protein genes the genomic RNA is not packaged and can not spread to other cells.

The alphavirus replicons have been used to express a variety of heterologous proteins and quantities in excess of 10 mg/10^8 cells have been reported *(18,22)*. These vectors are also proving to be important for studies of the virus life cycle. Infection of cultured cells by Sindbis virus and Semliki Forest virus leads to rapid inhibition of host cell protein synthesis and cytopathic effects (CPE) *(23)*. The mechanism(s) leading to the shutoff of the synthesis of host proteins is not known, but the viral structural proteins have frequently been implicated in this process (reviewed in ref. *24*). Studies with a variety of Sindbis virus replicons, however, showed that inhibition of host protein synthesis occurred under conditions in which no viral structural proteins or no subgenomic RNA were synthesized indicating that it is the early steps in viral gene expression that are responsible for the inhibition of host cell protein synthesis *(20)*. In contrast, Sindbis viruses and Sindbis virus replicons were clearly distinguished by the time at which CPE became evident. Viruses that synthesized high levels of the two membrane glycoproteins on the surface of the infected cells caused a rapid (12–16 h postinfection) appearance of CPE and those that did not synthesize the glycoprotein spikes showed delayed (30–40 h) CPE. Further studies with these replicons should define the step(s) in viral replication that lead to inhibition of host cell protein synthesis. (For reviews on the effects of virus infection on cellular protein synthesis see ref. *25*).

It has also been possible to insert heterologous amino acid sequences into the Sindbis virus structural proteins so that the virus retains viability and the heterologous epitope is expressed and functions as an immunogen. London et al. *(26)* were able to make random insertions of an epitope from Rift Valley Fever virus into the structural protein genes of Sindbis virus. Viable viruses containing the insert were then selected. The data showed not only that insertions were tolerated but also that the chimeric Sindbis virus could be used as a vaccine to make mice resistant to infection by Rift Valley Fever virus.

2.3. Plant Viruses

The plant virus brome mosaic virus (BMV) was the first example in which a foreign gene had been inserted into a self-replicating RNA. The genome of BMV consists of three RNA components: two of them, RNA1 and RNA2, are required for RNA replication. The third RNA, RNA3, encodes two genes, a 32 kDa protein, and the viral coat protein. The latter is translated from a subgenomic RNA transcribed from the (–) strand of RNA3. The cDNA of RNA3 was engineered to contain the CAT gene in place of the coat protein gene *(27)*. When RNA was transcribed from this cDNA and inoculated into plant protoplasts along with RNA1 and RNA2, the CAT protein was translated from the RNA3 subgenomic RNA. Other plant viruses including those with replication strategies similar to that of the alphaviruses have also been shown to be capable of tolerating and expressing foreign genes *(28–30)*. A major use of foreign genes in these systems has been as a tool for the analysis of viral gene expression. Several examples illustrate this point. Gallie et al. analyzed the regulatory effects of noncoding sequences (the 5' and 3' untranslated regions and the polyA tail) of tobacco mosaic virus in conjunction with different reporter genes and concluded that both the nontranslated and translated regions of a message can affect the level of expression *(31)*. Dolja et al. inserted the bacterial β-glucuronidase (GUS) gene into the potyvirus, tobacco etch virus, and used *in situ* histochemical GUS assays to visualize virus replication and movement in tobacco plants *(32)*. The theoretical yield of protein products encoded by this virus could be as high as 1% of the total detergent-solubilized protein in infected leaves suggesting that a chimeric virus such as this could be useful for the production of foreign proteins. In this regard, a yield of 2% of the total soluble protein was found when tobacco mosaic virus was used to express a foreign protein *(29)*. In the former example with the potyvirus, however, spontaneous deletion mutations in the heterolo-

gous sequences were observed indicating that those chimeric viruses were unstable *(33)*.

3. Negative Strand RNA Viruses as Vectors for the Expression of Heterologous Genes

Earlier in this article it was pointed out that the construction of infectious (–) strand RNA genomes from cDNAs has several hurdles that do not exist for the (+) strand RNA viruses. The major obstacle is that the isolated genomes of (–) strand RNA viruses are not infectious and can not be assayed for activity by direct transfection into cells. Although it has not yet been possible to make an infectious virus from the cDNA of (–) strand genomes, they are becoming tractable to genetic engineering and analysis. Several different strategies are being employed. Those developed for influenza virus and the paramyxoviruses have made use of a helper virus to provide the components necessary for the amplification of an altered viral RNA or ribonucleoprotein.

3.1. Influenza Virus and Paramyxoviruses

The genome of influenza virus is composed of eight different RNA molecules. Each RNA contains the coding information (in the opposite orientation) for one or in some cases two proteins. The first 12 nt at the 3' terminus and the first 13 nt at the 5' terminus are highly conserved among the different RNA segments and are also conserved among different strains of influenza virus type A (reviewed in ref. *34*). These conserved sequences function as recognition elements for replication, transcription, and packaging of the RNAs. Reverse genetics of an influenza virus gene involved the construction of a cDNA plasmid to contain the CAT gene—in the reverse orientation —flanked by 5' and 3' conserved sequences from the influenza virus genome. These sequences were placed downstream of the T7 promoter for in vitro RNA transcription. The transcribed RNA was not introduced directly into cells, but was first reconstituted into a ribonucleoprotein (RNP) using purified viral proteins *(35)*. The RNP was then transfected into cells that were also infected with influenza virus to provide the viral proteins required for replication, transcription, and packaging of the input RNP. These transfected–infected cells replicated and transcribed the chimeric RNA and translated the CAT mRNA *(34,35)*.

Paramyxoviruses are negative strand RNA viruses with a nonsegmented genome. It has been possible to engineer cDNAs derived from the genomes of at least two members of this family—Sendai virus *(36)* and respiratory syncytial virus *(37)*—for the expression of a foreign gene. The cDNAs consist of the CAT gene flanked by 5' and 3' noncoding sequences corresponding to those of the viral genome. In these examples, the RNA transcribed from the cDNA was transfected into cells that were infected with the homologous virus, unrelated viruses did not provide the required functions. The synthetic RNAs were transcribed, replicated and packaged into infectious virions. These systems will be of great value in identifying the cis-acting signals in the RNA genomes. Furthermore, respiratory syncytial virus is an important human pathogen and this type of vector may provide a tool for the development and characterization of attenuated vaccines.

3.2. Vesicular Stomatitis Virus

Studies with the rhabdovirus, vesicular stomatitis virus (VSV) provide another means of analyzing negative strand RNA viruses using recombinant DNA technology. Five viral proteins are required for transcription, replication, and assembly of the virus genome. The approach taken by Pattnaik et al. was to express each of these proteins individually from cDNA clones in which the viral genes were under the control of the T7 bacteriophage promoter *(38)*. These cDNAs were transfected into cells along with a cDNA containing a defective VSV genome also under the control of the T7 promoter. In addition, the cells were infected with a vaccinia virus containing the gene for the T7 polymerase. This infection–transfection system led to the amplification and assembly into particles of the defective VSV RNA—in the absence of any infectious VSV. A defective VSV RNA that contains heterologous sequences internal to the required terminal sequences was also amplified indicating that

this expression system may also have the potential to be developed into a vector *(38)*. It is now being exploited mainly to define the important sequences in the replication, transcription, and assembly of this virus.

4. Promises and Problems

Every new approach has a spectrum of exciting promises as well as potential difficulties and the use of RNA viruses as vectors for the expression of heterologous proteins is no exception. Two types of problems have to be considered. One problem is an obvious one—will they be useful? RNA genomes are highly mutable *(39)*. High mutation rates account for the phenomenon of antigenic drift in influenza viruses that is one of the reasons why the development of effective vaccines against influenza viruses has been difficult. Hepatitis C virus (a member of the flavivirus family) shows antigenic changes during the course of infection of an individual, and the ability of the virus to undergo rapid change may be one of the ways it is able to persist in the presence of an active immune response. The current infectious RNA virus vectors have shown some degree of instability *(9,16,33)* and this may limit the time in which a protein will continue to be expressed at a high level.

A second problem is the possibility of biohazards: What risks might arise with the use of infectious agents? One solution to this potential hazard will be the development of vectors that provide excellent delivery systems, but are not capable of spreading from cell to cell. The alphavirus vector systems may eventually fulfill this promise, but the present versions are probably not completely free of infectious particles. Furthermore, recombination between viral genomes might give rise to infectious particles. This has been one problem in the use of retroviruses *(40)*. Recombination has been observed in cells transfected with alphaviruses *(41)* and between viral RNAs and transgenic plant transcripts *(42)*. Although the latter might raise questions about plant engineering, a more reasoned view is that of Falk and Bruening who point out that "the potential benefits of engineered resistance genes far outweigh the vanishingly small risk of creating new and harmful viruses in significant excess over those being created by natural processes" *(43)*.

This is still an early stage in the development of RNA virus vectors and perhaps caution is wiser than enthusiasm. I have already mentioned some uses for these vectors. Some of these, as well as more speculative ones, will require a better understanding of the effects of these viruses on their hosts. Infection of cells with many RNA viruses, including picornaviruses, alphaviruses, and influenza virus, leads to inhibition of host cell protein synthesis and cell death. The ability to alter viral RNA genomes with the tools of recombinant DNA provides the means for studying these phenomena in more depth and perhaps altering the consequences of an infection. Then, genetic engineering could turn some viruses from agents of disease to agents of cure.

Acknowledgments

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