substrates and no packaging cell lines yet established in cell substrates likely to be approved for GMP vaccine production. These difficulties will be magnified in adapting these processes to commercial scale. Notwithstanding these issues, replicon vaccines derived from positive strand RNA viruses have shown tremendous promise in experimental systems and certainly merit continued effort to resolve these potential limitations for progression into the clinic.

A number of review articles have been published on expression systems derived from positive strand RNA viruses (92,167,174–192).

VACCINE VECTORS DERIVED FROM NEGATIVE STRAND RNA VIRUSES

Negative strand RNA viruses share advantages with positive strand viruses when configured as vaccine vectors. Both have evolved mechanisms for high-level protein expression, and neither directs the integration of foreign genetic material into the genome of the host. However, now that systems have been established for the efficient recovery of several negative strand RNA viruses from molecular clones and specific signals for gene expression have been defined, members of this group of viruses offer additional advantages. First, viruses with segmented genomes and/or filamentous nucleocapsids can more easily accommodate additional whole gene segments, or large gene insertions. Second, many are infectious by the intranasal (IN) route and vectors derived from them can deliver heterologous immunogens to the respiratory mucosa. Third, multiple serotypes exist in many cases, which facilitate effective booster strategies. Finally, in several cases, safe and effective vaccine strains have been reproduced as full-length cDNA clones and are available as well-documented starting points for development of vaccine vectors.

The biology of negative strand RNA viruses and their generation from cDNA clones has been extensively reviewed in Neumann et al. (193), and will be mentioned only briefly here.

Nonsegmented Negative Strand RNA Viruses

Several laboratories have developed and used reverse genetic systems to study the unique modular genome organization of this group of RNA viruses. These combined discoveries have enabled the use of several nonsegmented negative strand RNA viruses as vaccine vectors, and have launched a distinct field of study, extensively reviewed by Bukreyev et al. (194). The main points will be highlighted here.

Rhabdoviruses

The best-known members of the Rhabdovirus family are vesicular stomatitis virus (VSV) and RV, both of which have been exploited as vaccine vectors. Their genomic RNA is approximately 11 kb in length and encodes (in order from 3′–5′) nucleocapsid protein (N), phosphoprotein (P, previously NS, a cofactor in viral RNA synthesis), matrix protein (M), spike glycoprotein (G), and RNA-dependent RNA polymerase (L), all of which are found in the virus particle. The level of gene expression is directly related to gene position relative to the 3′ end of the RNP template, and rearrangement of the viral genes leads to slower growth in cultured cells and attenuation of virus virulence in the animal host (195). These rearranged genomes are stable on passage, as homologous recombination does not occur. There are three known serotypes of VSV, but only a single RV serotype. Specific mutations in both the VSV and RV glycoproteins have been shown to attenuate virulence (see below), and live attenuated vaccines for rabies are being used for oral immunization of wildlife (196). VSV has a wide host range, from insects to man, and causes a mild, flu-like illness in humans. Laboratory animals can be infected by VSV using subcutaneous, intracranial, or IN routes.

Schnell et al., working with RV, first demonstrated cDNA-based replication of a full-length recombinant rhabdovirus genome by using transfection of T7-driven RNA expression plasmids for the transcription of the complete genome complement (or antigenome) and N, P, and L mRNAs, followed by infection with vaccinia virus expressing T7 bacteriophage RNA polymerase (4). This strategy has been used successfully to produce recombinant VSV (197) and several paramyxoviruses (see below), and has been refined by replacing vaccinia virus with a T7-polymerase-expressing baby hamster kidney (BHK) cell line (198,199) or a cotransfected T7- polymerase expression plasmid (200,201). The demonstration of stable reporter protein expression from a sixth VSV gene, flanked by minimal start and stop signals and inserted between the G and L genes, followed soon after (202) (Fig. 1A.1). This basic strategy has been used in construction of numerous expression and vaccine vectors (203, also reviewed in Refs. 194,204–206), including those with two genes inserted into two different intragenic sites (207), or cocktails of two different vaccine vectors (208). Advantage has been taken of the signature 3′ to 5′ attenuation of transcription to modulate the level of gene expression. This was first shown with endogenous virus genes (209) and later with inserted heterologous genes (210,211). As predicted, the insertion of genes into more 3′ proximal intergenic sites leads to higher-level expression and a stronger immune response.

The ability of VSV to incorporate heterologous glycoproteins into its virions (212) suggested that heterologous glycoproteins expressed from VSV vectors would become part of analogous mosaic virions or pseudotypes. Viable viruses carrying additional genes for CD4, measles virus hemagglutinin (MH), or measles virus fusion protein (MF) contained varying amounts of the foreign protein inserted into their virion envelopes (203). The additional gene increased the length of the bullet-shaped particles, presumably due to increased length of the filamentous ribonucleocapsid, and reduced, to varying degrees, the level of virus replication in cultured cells. In many cases, but not all, no VSV-specific sequence was needed to drive incorporation of the additional glycoprotein into the virion envelope. The efficiency of VSV G incorporation into virions was not affected, suggesting that the new membrane protein occupied extra space in the envelope. By electron microscopy, each virus particle contained both proteins. This type of replication-competent VSV vaccine vector has been tested in animal models against measles virus (MV) (213), influenza virus (214,215), RSV (216,217), HIV-1 (208) bovine viral diarrhea virus (218), filoviruses (219,220), Lassa fever virus (221), SARS virus (222), and papillomavirus (210). Although the VSV vaccine vector expressing measles H protein displayed H protein on its surface, it nevertheless was able to induce protective levels of neutralizing antibody in the presence of maternal antibody, in contrast to the attenuated live virus measles vaccine (213). This result demonstrates the power of the recombinant vaccine vector approach in cases where vaccination in the presence of maternal antibody is needed to prevent dangerous early childhood infections.