I. Introduction

The segmented RNA genome of influenza virus is of negative polarity, i.e., the viral messenger RNA (mRNA) is complementary to the genome or virion RNA (vRNA) and the virion contains the enzyme system which transcribes the vRNA into the viral mRNA [75]. The synthesis of influenza viral mRNA involves a unique interaction with the host cell transcriptional machinery in the nucleus of the infected cell. This interaction is required first for the initiation of the synthesis of the viral mRNA chains. A viral endonuclease cleaves 5′-terminal fragments from newly synthesized capped (m'GpppNm-containing) cellular RNAs in the nucleus. These are most likely heterogeneous nuclear RNAs (hnRNAs), the precursors of cellular mRNAs [33, 42, 77]. These fragments of capped host nuclear RNAs serve as primers to initiate viral mRNA synthesis. The interaction with host cell nuclear functions apparently continues after the viral mRNAs are synthesized. The viral mRNAs, like other mRNAs (both viral and cellular) synthesized in the nucleus [81], contain internal N6 methyl adenosine (m6A) residues [43, 46], and several of the viral mRNAs appear to be generated by splicing like that occurring during the processing of hnRNAs to form cellular mRNAs [50]. Most likely, internal methylation and splicing of influenza viral mRNAs are carried out by cellular RNA processing enzymes in the nucleus.

Because the viral mRNAs contain host-derived sequences at their 5′ ends and lack sequences complementary to the last 17 to 22 nucleotides at the 5′ ends of the vRNA segments [29, 31, 42], the viral mRNAs are not suitable templates for the synthesis of vRNA (i.e., replication). The presumed templates for vRNA replication are full-length transcripts of the vRNA segments. These full-length transcripts, which lack 3′-terminal polyadenylate (poly A) sequences, comprise only 5% of the viral transcripts synthesized in the infected cell [29]. The synthesis of the fulllength transcripts requires the synthesis of one or more virus-coded proteins. The initiation of the full-length transcripts apparently occurs without a primer [32]. Also, the termination of transcription that occurs during viral mRNA synthesis 17 to 22 nucleotides from the 5′ end of vRNA must be prevented.
Very little is known about vRNA synthesis that is directed by the full-length transcripts. This chapter will review what is presently known about the synthesis of the three types of virus-specific RNAs—viral mRNA, full-length transcripts and vRNA—and will point out many of the remaining unanswered questions.

II. Viral mRNA Synthesis

A. Priming by Cellular Capped RNAs—Discovery

For many years, evidence accumulated indicating the influenza virus is unique among nononcogenic RNA viruses in requiring the functioning of host nuclear RNA polymerase II, the enzyme which synthesizes the precursors to cellular mRNAs [5, 52, 64, 85, 95]. The most definitive evidence was that α-amanitin, a specific inhibitor of RNA polymerase II, inhibits virus replication and that in mutant cells containing an α-amanitin-resistant RNA polymerase II, virus replication is also resistant to this drug [52, 85, 95]. The activity of RNA polymerase II was shown to be required for viral RNA transcription, even primary transcription: when added at the beginning of infection, α-amanitin inhibits all detectable viral RNA transcription [67].

An explanation for the RNA polymerase II requirement was proposed on the basis of studies of the transcription reaction catalyzed in vitro by the virion-associated transcriptase. It was found that the virion transcriptase is unable to initiate RNA synthesis effectively without the addition of a primer. In initial studies, specific dinucleoside monophosphates, ApG or GpG, at relatively high concentrations (about 0.2 mM) were found to act as primers to initiate chains [68, 78]. Transcription is strongly stimulated (as much as 100-fold) by ApG or GpG [68, 78], and the resulting viral RNA transcripts contain poly (A) and function as viral mRNAs in cell-free systems [11, 78]. With these dinucleotides, transcription initiates exactly at the UC sequence at the 3' ends of the vRNA segments [80, 93]. It was also found that the virion-associated transcriptase complex lacks enzymes capable of capping the 5' terminus of the viral RNA transcripts even when these transcripts contain di- or triphosphorylated 5' ends supplied by a ppApG or a pppApG primer, respectively [80]. However, it was known that, like most eukaryotic mRNAs [3, 91], viral mRNAs isolated from the infected cell do contain 5'-terminal methylated cap structures [46]. Based on these results, it was proposed that viral mRNA synthesis in vivo also requires a primer, but that the in vivo primer is not a dinucleotide but rather an RNA synthesized by RNA polymerase II, and that the 5' cap is derived from this primer RNA [78–80].

Direct evidence in support of this hypothesis was first obtained using the virion-associated transcriptase system. Initially, it was shown that the addition of purified β-globin mRNA to the virion-associated transcriptase stimulates viral RNA transcription in vitro about 80-fold [11]. On a molar basis, β-globin mRNA was found to be about 1000 times more effective as a primer than ApG. Subsequently, it was found that all RNAs tested that contain 5'-terminal methylated cap structures