Summary

Using fowl plague virus as a model highly virulent influenza A virus, we have studied the transcription and replication of the eight segments of the negative-stranded RNA genome. Initiation of transcription requires a 5' cap-containing RNA primer molecule which is recognised by PB2, the virion polypeptide product of RNA segment 1. During transcription, the 5' cap plus 10-15 nucleotides are transferred to influenza virus mRNAs, resulting in sequence heterogeneity at their 5' termini. Transcription is terminated by polyadenylation at a tract of uridine residues 17-22 nucleotides from the 5' terminus of each segment of the genome RNA template.

The site of RNA transcription appears to be the cell nucleus. Addition of toyocamycin to infected cells results in accumulation of virus-specific transcripts in the nucleus, and blocks the production of spliced mRNAs normally processed from transcripts of segments 7 and 8. Transcription is strictly regulated during normal replication, resulting in temporal control of polypeptide synthesis. This regulation can be abolished by cycloheximide, indicating that newly synthesised polypeptides (so far undefined) control transcription.

Little is known concerning the synthesis of progeny virion RNAs. Production of template cRNAs involves read-through of the polyadenylated site by an unknown mechanism. Since these cRNAs can be synthesised in the absence of new virion RNA synthesis, it is likely that only the input vRNAs are required to act as templates in their production.
INTRODUCTION

The influenza viruses are responsible for widespread epidemic disease in the human as well as avian, swine and equine species. In the two most recent pandemic years, 1957 and 1968, hundreds of millions of human cases occurred throughout the world with a high rate of excess mortality, and in the pandemic of 1918, more than twenty million persons died of acute influenzal pneumonia (1). The most virulent influenza virus so far isolated is the avian strain known as fowl plague virus (FPV), infection with which causes one hundred percent mortality in susceptible birds such as chickens (2). Our molecular studies of influenza virus replication have concentrated on FPV infection of chick embryo fibroblast (CEF) cells; this fully productive virus-cell system provides a model for the most virulent influenza infections in man.

Using FPV, we established that the influenza virus genome consists of eight distinct single-stranded RNA molecules (3) which are complementary to polysomal mRNA (4). Similar findings were made with other influenza virus strains (reviewed in 5). Subsequently, the sizes and coding assignments of the segments were determined (Table 1). The expression of influenza virus RNA segments involves transcription into capped, polyadenylated mRNAs (A(+)-cRNA) by a virion-bound RNA transcriptase. Production of genome RNAs, on the other hand, requires newly-synthesized virus-specific proteins and full-length non-polyadenylated template molecules (A(-)-cRNA). Although these reactions are extremely complex, many of the steps involved in influenza RNA biosynthesis are now understood at least in outline, and will be described here with reference to our studies using the fowl plague virus model.

EXPERIMENTAL

Initiation of mRNA synthesis studied in vitro

Initiation of transcription by fowl plague virion RNA transcriptase requires a primer molecule. This was originally suggested by the greatly enhanced rate of transcription observed when the dinucleotides GpG or CpC were added to an in vitro reaction