Quantification of Influenza Virus Messenger RNAs

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SUMMARY

The amounts of specific influenza virus cRNAs present on polyribosomes of cells treated with cycloheximide at various times after infection were measured by quantitative hybridization with $^{125}$I-labelled virus RNA segments. All species of cRNA were found associated with polyribosomes at every time point analysed. The relative abundance of the specific RNAs was in the same order as the reported relative synthesis of the influenza virus proteins. However, the concentrations of the cRNAs spanned a considerably smaller range than published protein synthetic rates, suggesting that both transcriptional and translational controls operate to regulate the final levels of influenza virus polypeptide synthesis.

INTRODUCTION

The genome of the WSN strain of influenza virus consists of eight distinct single-stranded RNA molecules which are transcribed in vivo to yield eight species of message RNA, the translation of which leads to the synthesis of eight or nine (Lamb et al. 1978) influenza virus proteins. The rates of synthesis of these virus-specific polypeptides are controlled throughout the infection cycle with the most abundant being made in approx. 50-fold greater amounts than the least abundant (Etchison et al. 1971; Lazarowitz et al. 1971; Skehel, 1972; Lamb & Choppin, 1976). It is clear from a number of studies that transcriptional controls are responsible for a significant portion of the differences in protein synthetic rates (Lamb & Choppin, 1976; Etkind et al. 1977; Hay et al. 1977) but at the present time it is not clear whether these controls are the only ones operating in this system.

In an attempt to further define the nature of the control of protein synthesis in influenza virus-infected cells, we have measured the relative amounts of individual influenza virus cRNAs available for translation at different times after infection. Influenza virus cRNA was prepared from polyribosomes of cells that had been briefly pre-treated with cyclo-heximide in order to saturate available cRNA with ribosomes. Under these conditions the RNA on polyribosomes should reflect the available mRNA (Lodish, 1976). The relative amount of each virus-specific cRNA was measured by following the kinetics of hybridization of infected cell polysomal RNA to highly radio-labelled individual vRNA probes. Throughout this paper we will refer to the polysome-associated virus cRNA as virus mRNA. We assume that the class of cRNA used as a template for the replication of vRNA is present in replicative intermediates and is therefore unavailable for recruitment into polyribosomes. This assumption is supported by the data of Hay et al. (1977) who reported only polyadenylated cRNAs associated with polysomes.

The results of the experiments reported here indicate (i) that the relative amounts of individual WSN influenza virus mRNAs differ over a fivefold range and (ii) that the ratios

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Fig. 1. Polyacrylamide gel electrophoresis of virion RNA of WSN strain of influenza virus. $^{32}$P-labelled influenza virus RNA, obtained from virus grown under high and low multiplicity conditions was prepared as described in Methods. Electrophoresis was conducted for 24 h at 30 mA in a 2.2% acrylamide gel containing 6 M-urea. The dried gel was exposed to X-ray film for 2 days. (a, c) RNA isolated from virions obtained from cells infected at a m.o.i. of 5; (b, d) RNA isolated from virions obtained from cells infected at a m.o.i. of 0.005. (c) and (d) contained twice as much RNA as did (a) and (b).

of individual mRNAs (with the exception of M mRNA) do not vary greatly during the infection.

METHODS

Virus and cells. The A/WSN/40 strain of influenza virus and the MDBK cell line were generously provided by Dr Purnell Choppin and were grown as described (Choppin, 1969). The MDCK cell line was obtained from the American Type Culture Collection and was propagated in 15 mM-N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) buffered Dulbecco’s modified Eagle’s medium (DME–HEPES) supplemented with 10% foetal bovine serum.

Preparation of individual influenza virus RNA hybridization probes. Confluent monolayers of MDCK cells were infected at multiplicities of 5 to 10 with MDBK-grown WSN virus. Infection medium, labelling procedures and purification of $^{32}$P-labelled virus were described previously (Privalsky & Penhoet, 1977). $^{32}$P-labellel virus RNA was purified as previously described (Tekamp & Penhoet, 1976). Electrophoresis of virus RNA was performed as described by Pons (1976). A typical separation of these RNAs is shown in Fig. 1. Occasionally, the top band was seen to separate into two poorly resolved species. Individual RNA
bands were located following a brief autoradiography. The RNA bands were excised and forced through a 25 gauge needle into 1 ml of 10 mM-tris-Cl, pH 7.4, 0.5 M-LiCl, 1 mM-EDTA, 0.5% SDS, 5% (v/v) phenol. Due to the incomplete resolution of the three largest RNAs, (those coding for the polymerase proteins; Palese et al. 1977) these RNAs were excised together. The RNA was eluted from the gel by shaking for 40 min at room temperature. Most of the acrylamide was pelleted by centrifugation for 10 min at 8000 rev/min in a Sorvall SS34 rotor. The RNA was precipitated with 2.5 vol. of 95% ethanol and overnight storage. The precipitated individual vRNAs were collected by centrifugation and dissolved in 10 mM-tris-Cl, pH 7.4, 100 mM-NaCl, 1 mM-EDTA and 0.2% SDS. They were purified further from contaminating acrylamide by centrifugation for 4 h at 50000 rev/min at 15 °C in an SW50.1 rotor in 15 to 30% sucrose gradients in the same buffer. Then 10 to 12 drop fractions were collected, the RNA located by Čerenkov counting and the appropriate fractions pooled. The purity of individual vRNA molecules was examined by re-electrophoresis which showed no evidence of cross-contamination. Further, the shapes of the Ct curves obtained using these probes (see below) were indicative of the complexities expected for single species.

The RNA was precipitated with ethanol, centrifuged and re-precipitated, was collected by centrifugation, and dried under nitrogen. Between 0.5 and 1.5 μg of a purified 32P-labelled vRNA was iodinated with 1 mCi of 125I (NEN) as described by Tereba & McCarthy (1973). Purified iodinated vRNA was dissolved in hybridization buffer (0.3 M-sodium acetate, pH 6.5, in 50% deionized formamide). Each iodinated vRNA had a specific activity of 1 x 10^6 to 3 x 10^7 ct/min/μg.

Preparation of 3H-labelled cDNA to influenza vRNA. The procedure followed was that of Taylor et al. (1977) scaled up five times. The labelled cDNA so prepared had a specific activity of 1 x 10^6 to 3 x 10^7 ct/min/μg and a modal sedimentation value of 5S.

Preparation of polyribosomal RNA. Confluent monolayers of MDCK cells on roller bottles were either infected at a multiplicity of 5 to 30 p.f.u./cell or mock infected. The addition of virus was taken as zero time. Before harvesting the cells, cycloheximide was added to a final concentration of 0.1 μg/ml and incubation at 37 °C was continued for another 10 min. All subsequent operations took place at 4 °C. Cells were quickly chilled by washing three times with ice-cold phosphate-buffered saline (Dulbecco & Vogt, 1954). Three to four ml of lysis buffer (50 mM-tris-Cl, pH 7.4, 10 mM-KCl, 5 mM-MgCl₂) plus 0.5% NP40 detergent plus 100 μg/ml each of heparin and cycloheximide were added to each bottle. Extracts were centrifuged for 10 min at 17000 g to pellet nuclei. The supernatant was removed, made 1% in Triton X-100 and layered on top of 15 to 50% (w/v) sucrose gradients in lysis buffer. The gradients were centrifuged for 3.5 h at 22000 rev/min at 2 °C in a Spinco SW25.1 rotor. Fractions of 1.5 ml were collected from each gradient and the absorbance at 260 nm was read. All fractions larger than the disome peak were pooled and precipitated with ethanol. The precipitated material was recovered by centrifugation and total polysomal RNA was extracted by the SDS-pH 9 phenol procedure described by Brawerman et al. (1972). The RNA was precipitated with ethanol, collected by centrifugation and re-precipitated. The re-precipitated RNA was collected by centrifugation, dried under nitrogen and finally resuspended in hybridization buffer to a final concentration of about 10 mg/ml.

Hybridization analysis. An amount of 3H-labelled cDNA or 125I-labelled vRNA in hybridization buffer was added to polyosomal RNA in the same buffer so that there were between 1000 and 2000 ct/min per sample to be taken. Depending on the amount of polyosomal RNA added this resulted in mass excesses [3 x 10^6 (for 2 h p.i. polyosomal RNA) to 2 x 10^6 (for 5 h p.i. polyosomal RNA)] of polyosomal RNA relative to the labelled probes. The volume of the annealing reaction was usually 55 μl which allowed for ten 5 μl amounts to be taken. The
hybridization reactions were carried out in 1 ml microfuge tubes (West Coast Scientific) previously sterilized by autoclaving. The reaction mixtures were heated for 3 min in a boiling water bath, quickly cooled in an ice bath and overlaid with mineral oil. At this time, a 5 μl amount was removed for a zero point and diluted into 1·1 ml of 2 × SSC (0·015 M-sodium citrate, 0·3 M-NaCl) (for RNA:RNA reactions) or into 1 ml 0·12 M-sodium phosphate buffer, pH 6·8 (for cDNA:RNA reactions) and immediately frozen in dry ice and stored at −20 °C until processed. The remainder of the reaction was put into a 37 °C water bath; samples were removed at appropriate times, diluted as described above and frozen until processed.

To assay the annealing of 125I-vRNA, each sample was split in half. One half was directly precipitated with an equal volume of 20% TCA in the presence of 100 μg of yeast RNA carrier. Ribonuclease A and T1 ribonuclease were added to the other half to a final concentration of 10 μg and 1 unit/ml respectively. After incubation for 20 min at 37 °C, carrier RNA and TCA were added as above. These digestion conditions had been worked out in trial experiments with 3H-labelled double-stranded virus RNA and excess cold yeast RNA and gave maximum discrimination between single- and double-stranded molecules.

To assay 3H-labelled cDNA:RNA hybrid formation, samples were brought to room temperature and applied to an approx. 5 ml column of hydroxylapatite [prepared as described by Siegelman et al. (1965)] maintained at 60 °C. The unbound (single-stranded) material was eluted with three 1 ml amounts of 0·12 M-sodium phosphate, pH 6·8, pre-equilibrated to 70 °C. The flow-through as well as the three washes were collected in one tube. The bound nucleic acid (duplex) was eluted with four 1 ml washes of 0·3 M-sodium phosphate buffer, pH 6·8, previously equilibrated to 70 °C. The 0·12 M eluate and 0·3 M eluate were made 10% in TCA after the addition of carrier RNA.

Trichloroacetic acid (TCA) precipitates were collected by filtration on to Whatman GF/C glass-fibre filters and were washed extensively with 5% TCA, followed by 95% ethanol. Filters were dried and 3H-labelled cDNA samples were counted in a toluene-based scintillant in a Packard scintillation counter. 125I samples were assayed in a Searle gamma-counter.

RESULTS

Preparation of influenza virus mRNA

The aim of the present study was to measure the amounts of individual virus message RNAs available for translation in influenza virus-infected cells. The most straightforward way to obtain a sample of message RNAs is to isolate it from polyribosomes. However, message RNA populations isolated from normal polyribosomes may be biased due to differences in efficiencies of initiation of protein synthesis on individual messages (Lodish, 1971, 1976; Lodish & Desalu, 1973). In order to minimize this bias, we have utilized cycloheximide in low amounts to slow the rate of protein chain elongation (Stanners, 1966; Lodish, 1971). Under these conditions, elongation becomes rate limiting and the polyribosomal mRNA population is much more reflective of the available mRNA pool (Lodish, 1976).

Fig. 2(a, b) presents comparisons of the polyribosome profiles obtained from influenza virus-infected MDCK cells harvested at 4 h p.i. either without (Fig. 2a) or with (Fig. 2b) a 10 min pre-treatment of cells with 0·1 μg/ml cycloheximide. As expected, cycloheximide treatment resulted in an increase in the number of ribosomes in polyribosomes and an increase in the average size of polyribosomes with a concomitant decrease in 80S monosomes. Thus, the influenza virus mRNAs isolated from polyribosomes prepared under these conditions should reflect the available mRNA pools.
Influenza virus messenger RNAs

Fig. 2. Effect of a low concentration of cycloheximide on polyribosomes. Two confluent bottles of MDCK cells (approx. $3 \times 10^7$ cells/bottle) were infected with influenza virus at a m.o.i. of 10. Four h later, cycloheximide was added to a final concentration of 0.1 µg/ml to one bottle. Cells were harvested 10 min later, cytoplasmic extracts were prepared and analysed as described in Methods. Centrifugation was from right to left. Arrows mark the position of the 80S monosome peak. (a) Cell not treated with cycloheximide. (b) Cells treated with cycloheximide.

Analysis of polyribosomal RNA for contaminating virus RNA

Polyribosome RNA isolated as described in Methods was analysed for contamination with virus RNA by hybridization of the polysomal RNA with a labelled cDNA probe complementary to virus RNA. The extent of annealing of this probe was determined by hydroxylapatite chromatography which fractionates duplex and single-stranded structures. The specificity of the probe was checked by annealing in the presence of excess virion RNA. As seen in Fig. 3(a) 85% of the labelled cDNA probe was driven into double-stranded structures by virion RNA (the data have been normalized to 100%). Large excesses of virion RNA produced no significant increases in the extent of hybridization, indicating that the failure to achieve complete hybridization was not due to low representation of some virus sequences. It seems most likely that the failure to reach 100% hybridization was due to the failure of the HAP columns to completely resolve partially double-stranded molecules.

Fig. 3 also presents an analysis of the annealing of labelled cDNA probe with polysomal RNA from representative infected and uninfected cells. Annealing was carried out to those
Fig. 3. Kinetics of annealing of \(^{3}H\)-labelled cDNA probe to influenza virus RNA and to polyribosomal RNAs. (a) \(^{3}H\)-labelled cDNA was annealed with an excess of virus RNA (2.6 \(\mu\)g/ml) for the times necessary to achieve the indicated values of \(C_{t}\). Samples were taken and the fraction of the probe that was annealed was determined by chromatography on hydroxylapatite. The data have been normalized to 100%; the actual extent of annealing was 85%. Annealing was performed as described in Methods. (b) Annealing of \(^{3}H\)-labelled cDNA probe to polyribosomal RNA prepared from infected and mock-infected cells was performed for the times necessary to achieve the indicated values of \(C_{t}\). Samples were taken and the percentage of the probe that was hybridized was determined by chromatography on hydroxylapatite. The different symbols refer to different polyribosomal RNA preparations and are as follows: ▲, Mock-infected; ●, 2 h p.i.; ○, 3 h p.i.; △, 4 h p.i.; □, 5 h p.i.

values of \(C_{t}\) (the concentration of RNA in mol nucleotide/litre \(\times\) time in seconds) determined to result in maximum hybridization of polysomal RNA with individual vRNA probe species. In this way, each polysomal RNA preparation was checked for contaminating vRNA. As can be seen, there was virtually no annealing of probe cDNA to polysomal RNA isolated at 2, 3, 4 and 5 h p.i. These data agree with the results of Nayak et al. (1976) who were unable to detect vRNA on polysomes by hybridization with excess cRNA. The procedure developed for preparation of polysomal RNA thus satisfies the requirement for vRNA-free polysomal RNA.

Hybridization of polyribosomal RNA to individual vRNA probes

Virus RNA probes labelled with \(^{125}\)I as described in Methods were individually annealed to large excesses of polysomal RNA isolated from mock-infected cells and infected cells at different times post-infection. Large excesses of polysomal RNA were used to ensure a high mass ratio of polysomal RNA to labelled probe vRNA. Early in infection mRNA species would be expected to be present in lowest amounts; typically the polysomal RNA:labelled probe ratio was \(3 \times 10^{6}\); at later times, mass ratios of \(10^{4}\) were used. The final extent of annealing was generally between 75 and 90%, never 100%, a phenomenon also reported by Taylor et al. (1977). Although we do not know the reason for failure to reach 100% hybridization it is not likely to be due to an insufficient concentration of mRNA sequences since it
Fig. 4. Hybridization of $^{125}$I-labelled vRNA probes to 2 h polyribosomal RNA. $^{125}$I-labelled vRNA probes were hybridized to polyribosomal RNA prepared 2 h p.i. (●—●) or mock infected (○—○). Numbers in each panel refer to the particular $^{125}$I-labelled vRNA used as probe. The data have been normalized to 100% annealing. The actual extent of annealing was: 1 to 3, 85%; 4, 70%; 5, 70%; 6, 75%; 7, 75%; 8, 72%. Annealing conditions are described in Methods. The final concentrations of infected cell polysomal RNA in the annealing reaction was 10–1 mg/ml for all reactions with the exception of that with species 8 vRNA where it was 10–3 mg/ml. The final concentration of uninfected cell polysomal RNA was 8.3, 8.1, 8.2, 7.9, 8.4 and 8.2 mg/ml for reactions with vRNA species 1 to 3, 4, 5, 6, 7 and 8 respectively.

was true also for polysomal RNA prepared late in infection when the proportion of influenza virus-specific mRNA sequences is high.

The earliest time that we measured individual virus mRNAs was at 2 h p.i. Fig. 4 is representative of the annealing kinetics for each vRNA probe with polysomal RNA from infected and mock-infected cells harvested at this time. In this figure, the data have been normalized to 100%; the actual extent of annealing of each probe species is given in the accompanying legend. Hybridization of individual labelled vRNA probes with polysomal RNA prepared from cells 3, 4 and 5 h p.i. produced very similarly shaped C, t curves and duplicate analyses showed very little variation (less than 10%). Table 1 represents a compilation of the measured C, t values of the various RNA species determined in these analyses.

**Analysis of hybridization data**

For the purposes of this study, we wished to measure the accumulation of individual influenza virus messages as a function of time after infection and to compare the relative amounts of the individual messages present at specific time points following infection. It is
Table 1. Comparison of the annealing of individual influenza RNA species at different times after infection

<table>
<thead>
<tr>
<th>RNA species</th>
<th>C_{T_{4}} at</th>
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<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>1-3</td>
<td>1900</td>
</tr>
<tr>
<td>4</td>
<td>980</td>
</tr>
<tr>
<td>5</td>
<td>1100</td>
</tr>
<tr>
<td>6</td>
<td>700</td>
</tr>
<tr>
<td>7</td>
<td>1000</td>
</tr>
<tr>
<td>8</td>
<td>480</td>
</tr>
</tbody>
</table>

* Final extents of annealing: 1 to 3, 77%; 4, 70%; 5, 75%; 6, 70%; 7, 80%; 8, 80%. Concentrations of polysomal RNA in annealing reactions: 1 to 3 - 0:28, 1:29 and 2:6 mg/ml; 4 - 0:28 and 2:65 mg/ml; 5 - 0:67, 1:3 and 2:9 mg/ml; 6 - 0:67 and 1:3 mg/ml; 7 - 0:28, 1:3 and 2:65 mg/ml; 8 - 0:28, 1:3 and 2:65 mg/ml.
† Final extents of annealing: 1 to 3, 86%; 4, 82%; 5, 75%; 6, 74%; 7, 88%; 8, 77%. Concentrations of polysomal RNA in annealing reactions: 1 to 3 - 0:27, 1:33 and 2:6 mg/ml; 4 - 0:27 and 2:64 mg/ml; 5 - 0:33, 0:67, 1:33 and 2:9 mg/ml; 6 - 2:14 mg/ml; 7 - 0:27, 1:32 and 2:64 mg/ml; 8 - 0:27, 1:31 and 2:64 mg/ml.
‡ Final extents of annealing: 1 to 3, 83%; 4, 82%; 5, 80%; 7, 88%; 8, 72%. Concentrations of polysomal RNA in annealing reactions: 1 to 3 - 0:32, 1:5 and 2:6 mg/ml; 4 - 0:32 and 2:63 mg/ml; 5 - 0:38, 0:68 and 1:34 mg/ml; 7 - 0:32, 0:67 and 2:63 mg/ml; 8 - 0:32, 1:33 and 2:63 mg/ml.

possible to estimate the amount of each mRNA on polysomes from the experimentally determined C_{T_{4}} values. The C_{T_{4}} is inversely proportional to the rate of hybridization (k): C_{T_{4}} \propto 1/k. The rate has been demonstrated to be directly proportional to the square root of the single-stranded mol. wt. (k \propto L^{0.5}) in the case where duplex DNA was sheared uniformly to different sizes and allowed to reanneal (Wetmur & Davidson, 1968). [However, when ribosomal RNA was sheared to average S values of 28, 9, 5 and 3:5 and annealed with complementary DNA on filters, the rate of hybridization was the same in all cases (Birnstiel et al. 1972).] The square roots of the length of the different 125I-labelled vRNA probes used in the current study were found to vary less than 1:5-fold. The square root of the lengths of the complementary RNA species varied less than twofold (calculated on the basis of vRNA mol. wt.). On this basis no correction was made for length of the reacting species; the effect, if any, of not making this correction would be a tendency to underestimate the amount of the larger species. The rate of hybridization is also inversely proportional to the complexity of the reacting species (Britten & Kohne, 1968; Bishop, 1969, 1972). In this study, there was a 10-fold difference between the complexity of the pool of species 1 to 3 and species 8. In order to calculate the relative amounts of different virus cRNAs, a complexity correction could be made on the basis of the mol. wt. of the various virus RNAs since each has a unique oligonucleotide sequence (McGeoch et al. 1976). If such a correction was applied to the data obtained, the resultant values would reflect the relative weight amounts of the various RNA species. However, to determine the relative molar amounts of the RNA molecules, the same algorithm would be applied in the opposite direction. Therefore, the experimentally determined C_{T_{4}} values themselves are indicative of the relative molar amounts of individual virus RNA messages.

Fig. 5 presents a series of accumulation curves (calculated from the data of Table 1) for most of the influenza virus message RNAs where the molar amount of each mRNA present at each time point has been normalized to the amount of species 4 mRNA present at 5 h p.i. For most of the RNAs, the maximal rate of accumulation appears to occur between 3 and 4 h p.i. Species 8 mRNA appears to continue to accumulate at a constant rate, while the rate of accumulation of species 7 mRNA increases late in infection. In most cases, these analyses were repeated several times with less than 10% variation from one experiment to another.
Influenza virus messenger RNAs

![Graph showing relative molar amounts of individual message RNAs present at various times after infection.](image)

Fig. 5. Relative molar amounts of individual message RNAs present at various times after infection. ●---●, 1 to 3; □—□, 4; △—△, 5; ▲—▲, 6; ○—○, 7; ■---■, 8. The molar amount of each RNA has been calculated relative to the amount of species 4 RNA present at 5 h p.i.

Table 2. Relative amounts of influenza virus cRNAs on polysomes at various times after infection

<table>
<thead>
<tr>
<th>Species of cRNA (protein product)</th>
<th>Time p.i. (h)</th>
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<tbody>
<tr>
<td></td>
<td>2  3  4  5</td>
</tr>
<tr>
<td>1–3 (P₁₋₃)</td>
<td>0.5 0.3 0.4 0.4</td>
</tr>
<tr>
<td>4 (HA)</td>
<td>1  1  1 1</td>
</tr>
<tr>
<td>5 (NP)</td>
<td>0.9 0.8 1.0 0.8</td>
</tr>
<tr>
<td>6 (NA)</td>
<td>1.4 1.4 0.8 ND*</td>
</tr>
<tr>
<td>7 (M)</td>
<td>1.0 1.1 0.6 2.1</td>
</tr>
<tr>
<td>8 (NS)</td>
<td>2.1 2.6 1.6 2.1</td>
</tr>
</tbody>
</table>

* ND, Not done.

Table 2 presents a comparison of the relative proportions of the virus mRNAs present at each time point analysed, normalized to the amount of species 4 mRNA present at that particular time. Tabulation of the data in this form emphasizes the fact that the relative amounts of the individual mRNAs stay constant throughout the infection, with the exception of species 7 (M) mRNA which increases late in infection as noted above.

DISCUSSION

In general, the rate of synthesis of a given protein is determined by the concentration of the specific message (this level is determined by control of transcription) and by the efficiency of its translation. The translation efficiency is, in turn, primarily dependent on the rate of initiation of protein synthesis on a given message (Fan & Penman, 1970; Lodish, 1971; Palmiter, 1972, 1974).

In cells infected with influenza virus, all eight virus proteins appear to be synthesized throughout the infection cycle (Lamb & Choppin, 1976; Privalsky & Penhoet, 1978).
However, the molar amounts of the individual virus proteins synthesized vary over a range of 50-fold or more with the P proteins being made in the smallest amounts and the NP, M and NS proteins being made in the largest amounts (Etchison et al. 1971; Lazarowitz et al. 1971; Skehel, 1972; Lamb & Choppin, 1976). To determine the kinds of controls operating in this system, a number of workers have measured the levels of individual message RNAs present in infected cells.

In several cases, estimates of individual message RNA concentrations were made by cell-free translation of RNAs isolated from infected cells (Lamb & Choppin, 1976, Inglis et al. 1978). Although not all of the virus proteins were detected in the in vitro translations, the data obtained in these studies showed that the in vivo and in vitro protein synthesis patterns were very similar, indicating significant controls at the level of transcription. In other cases, the levels of individual RNAs were assessed by hybridization techniques (Pons, 1977; Barrett et al. 1978). Barrett et al. (1978) performed quantitative hybridization analyses on oligo(dT)-selected RNAs. Their results also indicated some form of transcriptional control of polyadenylated cRNA production.

In the current study, we have performed an analysis of the levels of virus mRNAs available for translation by using the technique of brief cycloheximide treatment to saturate the RNAs with ribosomes (Lodish, 1976). At all time points analysed, the relative amounts of the various individual mRNAs present varied, generally reflecting the relative synthesis of the influenza virus proteins. This observation indicates that much of the control of specific synthesis of influenza virus proteins occurs at the level of transcription, a conclusion also reached in the studies discussed above and indicated in the direct analysis of mRNA synthesis reported by Hay et al. (1977). However, the data we have obtained in the present study indicate that the molar ratios of mRNAs present on polysomes vary only over a fivefold range. Since the reported molar ratios of the proteins synthesized vary approximately 50-fold, this suggests that the efficiency of initiation is higher on some influenza virus messages (e.g. M, NS and NP) than on others (e.g. P1-3) and that influenza virus protein synthesis is regulated by a combination of transcriptional and translational controls. Translational controls have also been observed in reovirus polypeptide synthesis in vivo (Joklik, 1974) and in vitro (Levin & Samuel, 1977) and in the synthesis of α and β globins and other non-reticulocyte polypeptides (Lodish, 1971; Lodish & Desalu, 1973). The availability of these sets of messengers should allow elucidation of at least some of the factors involved in the quantitative aspects of the initiation of protein synthesis in eukaryotes.

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REFERENCES


Influenza virus messenger RNAs


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