Cytoplasmic and Nuclear Input Virus RNPs in Influenza Virus-infected Cells

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SUMMARY

Chicken fibroblasts and MDCK cells were infected with influenza virus labelled with either 3H-uridine or 14C-amino acids, and the location in infected cells and properties of input virus-labelled structures were studied. Input virus RNA and protein were found in the cytoplasm of nuclei 1 h p.i. A part of the intranuclear parental structures was associated with chromatin while the other part could be extracted from nucleoplasm by 0·16 M-NaCl and represented free ribonucleoprotein (RNP) particles. These RNPs sedimented in glycerol velocity gradients at 40 to 70S, very similar to cytoplasmic RNPs, but differed distinctly from them in buoyant density. The bulk of cytoplasmic RNPs after fixation with formaldehyde banded in CsCl at 1·34 g/ml while nucleoplasmic RNPs banded at 1·39 or 1·41 g/ml. RNPs isolated from virions and infected cells contained the NP polypeptide which was revealed by SDS-PAGE analysis as a double band. The ratio of the two bands varied in cytoplasmic and nucleoplasmic RNPs, the lower band being dominant in cytoplasmic but not in nucleoplasmic RNPs. In addition, cytoplasmic RNPs were phosphorylated. The possible significance of intracellular RNP modifications for virus replication is discussed.

INTRODUCTION

Virus-specific ribonucleoproteins (RNPs) have been found in the cytoplasm and nucleus of influenza virus infected cells (Krug, 1972). They are involved in different functions during the replicative cycle. Besides their role as precursors of virion RNPs, they are associated with different complexes functioning in transcription and translation of virus-specific molecules. This complexity of functions implies the existence of several species of RNPs which could differ in their structure and composition. RNPs isolated from virions contain four polypeptides, the major structural unit NP and minor polypeptides P1, P2 and P3 which are presumably involved in polymerase activity. All the four polypeptides are revealed in RNPs isolated from the cytoplasm of infected cells which possessed polymerase activity (Inglis et al. 1976). Intracellular RNPs have been further separated into two subclasses one of which was enriched in P polypeptides (Caliguiri & Gerstein, 1978). The heterogeneity of intracellular RNPs in buoyant density has been demonstrated and the existence of RNP species of higher density than virion RNPs in the nuclei of infected cells has been described (Assadullaef et al. 1975; Caliguiri & Gerstein, 1978).

The input virus RNPs have been shown to penetrate into influenza virus infected cells and a significant fraction shown to enter the nucleus (Tenzov et al. 1977; Hudson et al. 1978). Since a growing amount of evidence suggests that transcription of the influenza virus genome initiates in the nucleus (see for example Taylor et al. 1977) the analysis of intra-
nuclear parental RNPs might be helpful for understanding the role of nuclei in early stages of the replicative cycle.

The present studies were undertaken to analyse the intracellular forms of input virus RNPs and in particular to investigate the intranuclear RNP particles, their location within the nuclei and their properties. We show that intranuclear RNPs differ distinctly from the cytoplasmic ones; they have higher buoyant densities in CsCl and their NP protein is modified.

**METHODS**

**Virus.** Influenza A<sub>0</sub> WSN (HoNI) was passaged in embryonated eggs at low multiplicity (10 ID<sub>50</sub>/egg) to avoid the accumulation of DI particles. Infectivity was determined by plaque assays performed on confluent monolayers of chicken embryo fibroblasts (CEF). Haemagglutinins were titrated with 1 % chicken erythrocytes. The egg-grown virus with a p.f.u./HA ratio of 10<sup>5.8</sup> to 10<sup>6.7</sup> was used as inoculum in preparing the radioactive virus.

**Radioactive virus.** Monolayer cultures of chicken fibroblasts (CEF) in 1 litre glass roller bottles were inoculated with allantoic virus at a multiplicity of 10 to 20 p.f.u./cell. After a 1 h adsorption period at 37 °C, 0.5 % lactalbumin hydrolysate supplemented with 2 % bovine serum (for <sup>3</sup>H-uridine labelling) or Hanks' solution supplemented with 0.01 M-HEPES, 10 % Eagle's medium and 2 % bovine sera (for <sup>14</sup>C-algae hydrolysate labelling) were added. After 2 h at 37 °C 100 μCi/ml of <sup>3</sup>H-uridine or 20 μCi/ml of <sup>14</sup>C-algae hydrolysate were added. In 24 h the medium was clarified by centrifugation for 10 min at 10,000 g at 4 °C and the virus was pelleted at 80,000 g through 25 % glycerol prepared in 0.01 M-tris-HCl buffer containing 0.01 M-EDTA. The virus pellet was resuspended in PBS Ca/Mg and layered on to a pre-formed 20 to 50 % glycerol gradient and centrifuged at 20,000 rev/min for 1 h at 4 °C in a Spinco SW41 rotor. The gradient was fractionated, the virus band collected and dialysed against PBS Ca/Mg overnight at 4 °C. The labelled virus had a p.f.u./HA ratio of 10<sup>5.0</sup> to 10<sup>5.2</sup>. Its sp. act. varied in the range from 1 to 5 ct/min/p.f.u.

**Infection of cells.** Confluent monolayers of CEF or MDCK (canine kidney) cells were inoculated with labelled virus at multiplicity about 1 p.f.u./cell. The virus was adsorbed at 4 °C for 1 h. The inoculum was removed and the cells were washed with growth medium to remove residual unadsorbed virus. The cells were then brought to 37 °C by the addition of warm medium (lactalbumin hydrolysate) and incubated at 37 °C for 30 or 60 min. The monolayers were then rinsed with cold Hank's solution and the cells harvested by scraping with glass beads.

**Cell fractionation.** Cells were washed with reticulocyte standard buffer (RSB; 0.01 M-tris-HCl, pH 7.4; 0.01 M-KCl; 0.0015 M-MgCl<sub>2</sub>). After 10 min at 0 °C they were broken by 20 to 25 strokes of a close fitting Dounce homogenizer and the nuclei were pelleted at 1000 g for 2 min. The supernatant was designated 'cytoplasmic fraction' and was further fractionated by centrifugation at 15,000 g for 15 min. This supernatant (S<sub>15</sub>) was designated 'cytoplasmic extract'. The nuclei were purified by repeated washing with 1 % Triton X-100 in RSB and then by centrifugation through 2 M-sucrose prepared in RSB containing 0.001 M-CaCl. The purity of the nuclei was checked by electron microscopy (Fig. 1) and by titration of haemagglutinins in the nuclear fraction (the purified nuclei contained less than 1 % of the HA associated with infected cells). For further fractionation of the nuclei, they were resuspended in 0.01 M-tris, pH 8.0, 0.16 M-NaCl, 0.001 M-MgCl<sub>2</sub> and the RNPs from the nucleoplasm were extracted by vortexing at 4 °C. Two or three such extractions were performed with intermittent centrifugation to pellet the nuclei. After these extractions, the chromatin was isolated by one of two methods: (i) the nuclei were treated with deoxy-ribonuclease I (100 μg per 10<sup>7</sup> nuclei) at 37 °C for 15 min and then the chromatin was
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Fig. 1. Electron micrograph of CEF nuclei purified as described in Methods.

extracted by high salt buffers, 0.5 M-NaCl, 0.01 M-tris, pH 7.4, 0.005 M-MgCl₂, 0.003 M-dithiothreitol followed by 2.0 M-NaCl, 0.01 M-tris, pH 7.4, 0.005 M-MgCl₂, 0.003 M-dithiothreitol at 4 °C, (ii) the nuclei were treated with deoxyribonuclease I, then resuspended in 0.001 M-EDTA, pH 8.0, and soluble chromatin was isolated after 30 to 40 min at 4 °C. The insoluble chromatin was extracted by the buffer containing 2.0 M-NaCl used in Method (i). The residual fraction was pelleted at 1000 g.

Glycerol gradient centrifugation. Cytoplasmic or nucleoplasmic extracts were layered onto pre-formed linear 15 to 30% (w/w) glycerol gradients prepared in TNE buffer (0.01 M-tris, pH 7.4, 0.1 M-NaCl, 0.001 M-EDTA) and centrifuged for 16 h at 18000 rev/min at 4 °C in a Spinco SW 41 rotor. The gradients were fractionated and acid-insoluble radioactivity in the fractions was determined.

Buoyant density analysis. The fractions of the glycerol gradients were fixed with 4% formaldehyde and layered on to linear CsCl gradients prepared in PBS containing 0.01 M-NaCl, 0.001 M-EDTA and 4% formaldehyde. The gradients were centrifuged at 4 °C for 14 h at 35000 rev/min in the SW 50 rotor. The gradients were then fractionated, refractive indices determined and radioactivity counted in acid-insoluble material in toluene based scintillation fluid in a Packard scintillation counter.

SDS-PAGE. Samples were heated for 2 min at 100 °C in 2% sodium dodecyl sulphate
(SDS), 5% mercaptoethanol and 0.1 M-tris buffer, pH 6.8, and analysed by electrophoresis in polyacrylamide slab gels containing SDS according to Laemmli (1970). Autoradiograms were prepared from dried gels.

**Tryptic peptide maps.** Virus proteins were separated on 7.5% polyacrylamide slab gels. The proteins were stained with Amido black and both bands of NP were sliced from the gel with a razor blade. The slices were washed with 25% isopropyl alcohol, then with 10% methanol and then dried. The proteins were then radio-iodinated with 125I in the gel slice by the chloramine T method according to Elder *et al.* (1977). After washing with 10% methanol, the slices were dried, digested with trypsin (50 μg/ml, Worthington, 271 units/ml) overnight at 37°C, then lyophilized. The digests were separated in two dimensions by electrophoresis at pH 4.7 toward the cathode with the origin on the left, followed by ascending chromatography using buffer I (acetic acid:formic acid:water, 15:5:80) for electrophoresis and buffer II (butanol:pyridine:acetic acid:water, 32.5:25.5:20). The plates were dried overnight and exposed to the film for 24 h.

**Chemicals and isotopes.** All chemicals used for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif., U.S.A. 3H-uridine (sp. act. 25 Ci/mmmol) was purchased from Radioactive Centre, Leningrad, 14C-algae hydrolysate (1 mCi/1 mg) from Prague, Czechoslovakia. Dithiothreitol and deoxyribonuclease I were purchased from Calbiochem, U.S.A.

**RESULTS**

*Localization of input virus components in infected cells*

The monolayers of CEF and MDCK cells were infected with WSN labelled with 3H-uridine or 14C-amino acids, the cells were fractionated 1 h after incubation at 37°C and input virus radioactivity in cellular subfractions was determined. A typical set of data is shown in Table 1. It can be seen that input virus RNA and protein are present in the cytoplasm and nuclei, their amounts in the nuclei being half or more of the total cellular radioactivity for MDCK cells and about 40% for CEF. In the control CEF where the labelled virus was added prior to cell homogenization, the percentage of nucleus associated label was insignificant.

To study the intranuclear localization of input virus components the nuclei were further fractionated as described in Methods. As shown in Table 2, part of the intranuclear radioactivity was extracted from the nuclei by isotonic salt buffer (nucleoplasmic fraction). The remaining radioactivity was extracted by high ionic salt buffers (Method i) and was distributed between soluble and insoluble chromatin (Method ii). Analysis of this material has shown the stable association of input virus RNPs with cellular chromatin (Bukrinskaya *et al.* 1979). The residual pellets contain smaller amounts of input virus radioactivity and have not been further analysed.

*Biophysical characterization of cytoplasmic and nucleoplasmic subviral components*

Centrifugation of cytoplasmic extracts in 15 to 30% linear glycerol gradients revealed the bulk of input virus RNA and protein in the 40 to 70S region of the gradient, i.e. in the region expected for influenza virus RNPs. The radioactivity after centrifugation of nucleoplasmic extract was distributed similarly [Fig. 2i (b) and 2ii (b)]. However, differences between cytoplasmic and nucleoplasmic RNPs were revealed when the peak fractions from glycerol gradients were re-centrifuged in CsCl. Cytoplasmic RNPs banded heterogeneously, the bulk of radioactivity being found at 1.34 g/ml, the density expected for influenza virus RNPs (Gitelman & Bukrinskaya, 1971; Krug, 1971), and smaller components were revealed at 1.39 to 1.41 g/ml [Fig. 2i (c) and 2ii (c)]. Meanwhile, the bulk of nucleoplasmic...
Table 1. The localization of labelled input virus components in infected cell subfractions 1 h p.i.

<table>
<thead>
<tr>
<th>Cell subfractions</th>
<th>MDCK cells*</th>
<th>CEF*</th>
<th>Uninfected CEF + virus†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3H-virus</td>
<td>14C-virus</td>
<td>3H-virus</td>
</tr>
<tr>
<td></td>
<td>ct/min %</td>
<td>ct/min %</td>
<td>ct/min %</td>
</tr>
<tr>
<td>Cytoplasmic extract</td>
<td>115300</td>
<td>44.7</td>
<td>113600</td>
</tr>
<tr>
<td>Nucleus</td>
<td>142560</td>
<td>55.3</td>
<td>109400</td>
</tr>
</tbody>
</table>

* Cell monolayers were infected with the virus labelled with 3H-uridine or 14C-amino acids and incubated at 37 °C. At 1 h p.i. the cells were removed from the glass and fractionated, the nuclei purified and input virus label determined in acid-insoluble material.
† Uninfected cells were collected and labelled viruses were added prior to fractionation.

Table 2. The localization of labelled input virus components in nuclear subfractions

<table>
<thead>
<tr>
<th>Method (i)</th>
<th>Method (ii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCK cells</td>
<td>CEF</td>
</tr>
<tr>
<td>Nuclear subfractions*</td>
<td>3H-virus</td>
</tr>
<tr>
<td></td>
<td>ct/min %</td>
</tr>
<tr>
<td>0.16 M-NaCl</td>
<td>98800</td>
</tr>
<tr>
<td>0.5 M-NaCl</td>
<td>32100</td>
</tr>
<tr>
<td>2.0 M-NaCl</td>
<td>97050</td>
</tr>
<tr>
<td>Residual pellet</td>
<td>19350</td>
</tr>
<tr>
<td>Nuclear subfractions*</td>
<td>ct/min %</td>
</tr>
<tr>
<td>0.16 M-NaCl</td>
<td>127600</td>
</tr>
<tr>
<td>Soluble chromatin</td>
<td>92500</td>
</tr>
<tr>
<td>2.0 M-NaCl</td>
<td>106800</td>
</tr>
<tr>
<td>Residual pellet</td>
<td>42200</td>
</tr>
</tbody>
</table>

* Cell monolayers were infected and fractionated as indicated in Table 1. The nuclei were further fractionated by one of two methods described in Methods. Each column shows the results of separate experiments.

RNPs banded at higher densities. The nucleoplasmic RNPs from MDCK cells banded as a single peak at 1.41 g/ml [Fig. 2ii (d)]. The bulk of nucleoplasmic RNPs from CEF banded at 1.39 g/ml and a minor fraction at 1.41 g/ml [Fig. 2i (d)]. The higher buoyant density of nucleoplasmic RNPs suggests a higher RNA to protein ratio as compared to cytoplasmic RNPs.

Intracellular input virus polypeptides

For further characterization of intracellular virus components, the input virus polypeptides from the cells infected with 14C-amino acid labelled virus were analysed by SDS-PAGE. All the virus polypeptides were found in the cytoplasmic fraction. When this material was fractionated by centrifugation at 15000 g to sediment membranes and mitochondria, all the virus polypeptides were present in the pellets while the supernatant (cytoplasmic extract) contained the single polypeptide, the major RNP polypeptide NP (Fig. 3a). NP was discovered in the pellets after centrifugation of cytoplasmic extracts at 120000 g (Fig. 3a, lane 4), suggesting that this polypeptide is associated with virus RNP and is not a soluble protein. We failed to detect P proteins, possibly owing to insufficient radioactivity in the samples.

The double band in the position of NP was clearly seen in most of the samples of RNPs isolated from virions and infected cells. To show that both bands have similar oligo-
peptides, radioiodinated samples were analysed by tryptic peptide mapping according to
the method of Elder et al. (1977). It can be seen from Fig. 4 that both bands share the same
peptides, confirming our suggestion that they are both NP protein. However, when NP
labelled with $^{14}$C-amino acids was analysed, at least three spots were found to be greatly
diminished in intensity or missed in the polypeptide in the lower band as compared to that
in the upper band (unpublished data) suggesting that this polypeptide may represent the
product of NP partial proteolysis.

The upper band was dominant in NP of WSN virions as well as in RNPs accumulated in
the cytoplasm in the late stages of infection (Fig. 5). Meanwhile, the lower band became
much more intense in input virus RNPs isolated from infected cell cytoplasm (Fig. 3a).
To determine whether the modification of NP occurs in the cytoplasm or on the plasma
membranes, we analysed the proteins of labelled virions after contact with the cells. As
shown in Fig. 3(b), the lower band of NP became more intense in this case, the results
depending on the cell concentration and temperature of adsorption. It is also seen from
Fig. 3(b) that M protein is modified after contact with cells and that some of the molecules
migrate more rapidly.

The phosphorylation of NP molecules, has been described for WSN virions and newly
synthesized RNPs by Privalsky & Penhoet (1977, 1978). To determine whether the input
virus NP was similarly modified in infected cells, CEF cells were infected with unlabelled
virus with multiplicity of infection about 100 p.f.u./cell, treated with cycloheximide (100 μg/
ml) just after infection to prevent the synthesis of virus-specific proteins and, at 30 or 60 min
after incubation at 37 °C, exposed to $^{32}$PO$_4$ for 30 min. The cells were then fractionated and

Fig. 2. Centrifugation of cytoplasmic and nucleoplasmic extracts from CEF and MDCK cells
infected with $^3$H-uridine-labelled influenza virus in linear glycerol and CsCl gradients. Infected
cells were incubated for 1 h at 37 °C, then disrupted by Dounce homogenization. The cytoplasmic
fraction was centrifuged at 15000 g for 15 min and the supernatant (cytoplasmic extract) was
used. The nuclei were purified and nucleoplasmic RNPs were extracted by 0.16 M-NaCl. Cyto-
plasmic and nucleoplasmic extracts were centrifuged in a 15 to 30 % glycerol gradients prepared
in TNE buffer and processed for acid-insoluble radioactivity determination. The peak fractions
(bracketed) were diluted 1 : 1 with phosphate buffer containing 0.01 M-EDTA, treated with 4 %
formaldehyde and centrifuged in pre-formed CsCl gradients prepared in the same buffer as de-
scribed in Methods. The gradients were fractionated and processed for acid-insoluble radioactivity
and buoyant density determinations. Panel (i), CEF; panel (ii), MDCK cells: (a), (b) glycerol
gradients, (c), (d) CsCl gradients, (a), (c) cytoplasmic extracts, (b), (d) nucleoplasmic extracts.
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Fig. 3. Modification of 14C-labelled NP protein in infected cell cytoplasm (a) and in WSN virions eluted from the cells (b). Panel (a) CEF were infected with 14C-labelled influenza virus. At 1 h p.i. the cells were fractionated by Dounce homogenization, the cytoplasmic fraction was centrifuged at 15,000 g and the supernatant (cytoplasmic extract) was further centrifuged at 120,000 g. The samples were analysed by autoradiography after electrophoresis in a 10% polyacrylamide-SDS slab gel. Lane 1, purified influenza virus; lane 2, P15 fraction; lane 3, S15 fraction (cytoplasmic extract); lane 4, the pellet after centrifugation of the cytoplasmic extract at 120,000 g. Panel (b) 14C-labelled influenza virus was collected after adsorption for 1 h on CEF monolayers containing 5 x 10^6 (lanes 1, 2, 3) and 2 x 10^7 (lanes 4, 5) cells (m.o.i. 2 and 0.5 p.f.u./cell respectively, the vol. of virus being 1 ml per 0.5 and 1.5 1 flasks). Electrophoresis was performed in a 7.5% polyacrylamide SDS slab gel. Lane 1, adsorption at 4 °C; lanes 2 and 4, adsorption at 20 °C, lanes 3 and 5, adsorption at 37 °C.

the cytoplasmic extracts subjected to SDS-PAGE. The band of phosphoprotein in the cytoplasmic extract from infected cells in the position of NP was seen in both cases, when ^32P was added at 30 min and at 60 min p.i., but was not found in material from mock-infected cells (Fig. 6a, b). These findings suggest that input NP is phosphorylated in the cytoplasm. In a number of experiments, the ^32P-labelled band coincided with the lower band of 14C-labelled NP (see below and Fig. 6b).

When the crude nuclear fraction was analysed by SDS-PAGE, the polypeptides HA, NP and M were revealed in autoradiographs (Fig. 7a). NP is not resolved into two bands in this gel, M polypeptide is revealed in trace amounts and the polypeptides of higher
Fig. 4. Comparison of the tyrosine-containing tryptic peptides of upper (a) and lower (b) bands of NP from the virions radioiodinated within the gel slices. Both bands in the position of NP were sliced from the stained gel shown in Fig. 5. The slices were treated, radioiodinated and analysed as described in Methods.

mobility possibly representing broken molecules of M or HA proteins are seen. The purified nuclei as well as all the nuclear subfractions and the structures sedimenting in the 40 to 70S region of glycerol gradients (see Fig. 2i, ii) contained the single virus polypeptide NP, suggesting that input RNPs released from virus envelopes enter the nuclei. The ratio of the two bands in nuclear NP was changed as compared to NP in the cytoplasm, since the upper band was more intense (Fig. 7b, c). 32P-labelled proteins were not found in the nuclei.

DISCUSSION

The results presented show that input virus RNPs penetrate influenza virus (WSN) infected cells and are present in cytoplasmic extracts and nuclei thus confirming our previous data made with FPV (Tenzov et al. 1977). In general, these results are in accordance with the data of Hudson et al. (1978) who have shown that labelled input RNPs of FPV penetrate the infected cells at 4 °C and a large proportion of them is associated with nuclei. However, there are some discrepancies which could be explained by the fact that the experiments were carried out at different temperatures. Hudson et al. (1978) found some M protein in purified nuclei while we detected only NP protein (P proteins were not detected in any fraction because of insufficient labelling). They also reported that input RNPs were not associated with chromatin while in our experiments RNPs were extracted with chromatin and, as described elsewhere (Bukrinskaya et al. 1979), were stably associated with it. The functions of the chromatin-associated RNPs and their relations to free RNPs in the nucleoplasm are unknown. Both species of RNPs could represent dynamic structures: for example, free RNPs could be precursors of chromatin-associated particles (or the bound particles are released and accumulated in the nucleoplasm).

As shown here, the input virus RNPs which penetrate the cell are modified and nucleo-
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Fig. 5. Polypeptides of WSN and of virus RNPs accumulated in the cytoplasm late in infection. WSN was purified as described in Methods. CEF were infected at a m.o.i. of about 100 ID₅₀/cell, the cytoplasmic extracts were obtained 20 h p.i. and centrifuged in 30 to 60% linear sucrose gradient prepared in RSB, in a Spinco SW41 rotor at 35000 rev/min for 16 h. The fractions containing virus RNPs were analysed by SDS-PAGE. The gel was stained with Amido black. Lane 1 RNPs from the cytoplasm of infected cells 20 h p.i.; lane 2, WSN cultivated in CEF; lane 3, WSN cultivated in chick embryos.

Fig. 6. Input virus polypeptides labelled with ¹⁴C-amino acids or ³²P in infected cells. CEF were treated with cycloheximide (100 µg/ml) for 1 h, infected with ¹⁴C-labelled or unlabelled WSN and the same medium with cycloheximide was added 30 min p.i. (a) or 60 min p.i. (a, b). After incubation at 37 °C the cells infected with unlabelled virus were exposed to ³²P for 30 min. Cytoplasmic extracts and purified nuclei were obtained and analysed by SDS-PAGE in 10% polyacrylamide slab gels. (a) Lane 1, cytoplasmic extract from infected CEF labelled with ³²P from 30 to 60 min p.i.; lane 2, cytoplasm from CEF infected with ¹⁴C-labelled WSN; lane 3, cytoplasmic extract from infected CEF labelled with ³²P from 60 to 90 min p.i. (b) Lane 1, cytoplasmic extract from infected cells labelled with ³²P from 1 to 1.5 h p.i.; lane 2, purified nuclei from CEF 1.5 h p.i.; with ¹⁴C-labelled virus; lane 3, cytoplasmic extract from mock-infected cells labelled with ³²P for 30 min.

Nuclear RNPs differ distinctly from the cytoplasmic RNPs in a number of properties. Though they sediment similarly in velocity glycerol gradients, the density of nucleoplasmic RNPs in CsCl is higher than that of cytoplasmic ones. The bulk of cytoplasmic RNPs has the same density as the nucleocapsids isolated from virions (ρ = 1.34 g/ml), whereas RNPs isolated from the nucleoplasm of infected MDCK cells have a density of 1.41 g/ml and from
Fig. 7. Input virus polypeptides in CEF (a, b) and MDCK cells (c) infected with 14C-labelled virus. At 1 h p.i. the cells were fractionated, the nuclei purified and further fractionated by Method (i) as described in Methods and polypeptides analysed by SDS-PAGE in 10 % (a, b) or 7.5 % (c) slab gels. (a) Lane 1, purified WSN; lane 2, crude nuclear pellet before purification; lane 3, purified nuclei. (b) NP region of the gels; lane 1, cytoplasm; lane 2, 0.5 M-NaCl extract. (c) Lane 1, cytoplasm; lane 2, the pellet after centrifugation of the cytoplasmic extract at 120000 g; lane 3, the nucleoplasmic structures extracted by 0.16 M-NaCl sedimented at 40 to 70 S region of a glycerol gradient; lane 4, purified nuclei; lane 5, purified WSN; lane 6, the pellet after centrifugation of 0.16 M-NaCl nuclear extract at 120000 g.

CEF 1.39 and 1.41 g/ml. The differences in RNP densities in both cell lines are not understood. One may speculate that the higher density of nucleoplasmic RNPs in MDCK cells is somehow connected with the higher yield of influenza virus produced by these cells.

The increase in buoyant density suggests the loss of part of RNP proteins. It could also mean a conformational modification of RNP structure. Both these processes might occur in infected cell nuclei; for example, RNPs may lose the part of the protein which is essential for spiralization and this may be followed by uncoiling of the tightly spiralized nucleocapsid thread. The suggestion that the RNPs with density 1.41 g/ml represent uncoiled nucleocapsids was confirmed by electron microscopic observations made by S. Klimenko (unpublished data). In connection with the high buoyant density of nucleoplasmic RNPs, the input RNPs resemble the RNPs newly synthesized in infected cells which have the same buoyant density in CsCl (ρ = 1.41 g/ml; Assadullaeff et al. 1975). Newly made RNPs have been recently separated into two subclasses by isopycnic centri-
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fugation, one subclass having a buoyant density of 1.26 g/ml and the other of 1.12 g/ml in renograin suggesting their different RNA to protein ratio. The subclass with the higher RNA content is enriched with P proteins and is suggested to be involved in RNA synthesis (Caliguiri & Gerstein, 1978). Similarly, it could be suggested that intranuclear input virus RNPs of high density represent RNP complexes involved in primary transcription.

The post-translational modification of proteins such as cleavage and phosphorylation-dephosphorylation are known to represent on-off switches for the activity of many enzymes and regulatory proteins (Uy & Wold, 1977). Both these processes seem to occur in influenza virus infected cells in connection with the main structural RNP protein, NP. We found two distinct forms of NP in virions and infected cells which slightly differed in their migration rates in SDS-polyacrylamide gels apparently due to the partial proteolysis of NP molecules. The proteolysis and phosphorylation of NP appear to take place in the cytoplasm (possibly on plasma membranes) but not in the nuclei, where predominantly uncleaved and possibly dephosphorylated NP is found.

Further study of intranuclear RNP modifications seems to be important. It may give some clues as to the role of the nucleus in the initiation of infection. For example, these modifications could facilitate correct transcription of the virus genome and the interaction of the genome with molecules of host RNA which function as primers in virus transcription (Bouloy et al. 1978).

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REFERENCES


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