# A DIRECT APPROACH TO STUDY THE MESSENGER PROPERTIES OF INFLUENZA-VIRION RNA

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## 1. Introduction

For several animal viruses, containing a single stranded RNA genome, it is still unknown if the virion type RNA or the complementary strand, synthesized only after infection, functions as messenger. This applies also to the influenza virus [1, 2].

A direct way to solve this problem is provided by adding viral RNA to an *in vitro* protein synthesizing system and by examining if virus specific proteins are made.

Recently we have been able to show that RNA from avian myeloblastosis virus (AMV) can be translated faithfully in a heterologous *in vitro* system for protein synthesis prepared from *E. coli* cells [3]. There is evidence that the same is true for the RNA of other C-type particles [4, 5].

Here we present evidence that this system also can successfully be used to study the messenger properties of influenza virion RNA. We can demonstrate that this RNA is translated into several proteins, which correspond to structural proteins of the virion with respect to their molecular weights.

## 2. Material and methods

The preparation of the cell-free system [6], the incubation conditions [3, 6], the extraction of the viral RNA [3] and the analysis of the *in vitro* product [3, 6, 7] have been described recently. For the labelling of the *in vitro* proteins L-[$^3$H]histidine (specific activity 50 Ci/mM), purchased from the Radiochemical Centre (Amersham, England), was used.

## 3. Results and discussion

The addition of RNA extracted from influenza virions to the cell-free system gives rise to a high incorporation of amino acids into TCA-precipitable polypeptides, comparable with the stimulation obtained under the direction of phage M 12 RNA.

Influenza RNA directs the incorporation of 26 000 cpm of [$^3$H]histidine per 10 µl reaction mixture during an incubation of 20 min at 37°C. In comparison, using the same conditions, 35 000 cpm were obtained with phage M 12 RNA, versus 1000 cpm without addition of template.

To see if the stimulation observed was due to the synthesis of virus specific proteins, the *in vitro* product was analysed on SDS-polyacrylamide gels (fig. 1). The upper curve shows a densitometer profile of a stained gel loaded with influenza proteins. The lower curve represents the radioactivity pattern of a gel containing proteins synthesized *in vitro* in presence of [$^3$H]histidine.

They demonstrate that influenza RNA directs the synthesis of high molecular weight proteins, which coelectrophorese with the virion proteins.

There are radioactivity peaks corresponding to the viral polypeptide P, the nucleoprotein (NP), the haemagglutinin subunits ($HA_1$, $HA_2$) the neuraminidase (NA) and probably also for the protein M.

However, there is faster migrating material, which may reflect the synthesis of the nonstructural protein (NS) [8] or just represent incomplete polypeptide chains.

In addition to the facts already presented we are able to show by immunodiffusion and precipitation

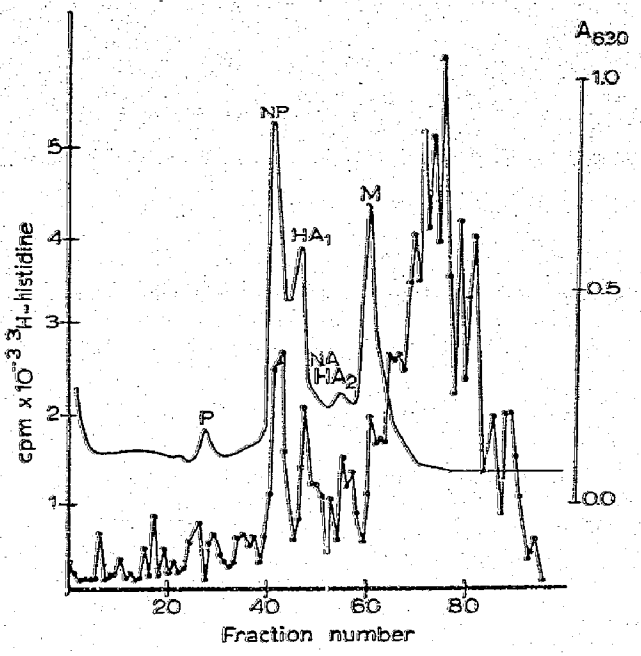


Fig. 1. Analysis by SDS-polyacrylamide gel electrophoresis of [$^3$H]histidine labelled cell-free product. Upper curve: densitogram of a stained gel loaded with 150 μg of unlabelled influenza APR 8 proteins (——); Lower curve: radioactivity pattern of a gel containing about 150 000 cpm of [$^3$H]histidine labelled proteins synthesized *in vitro* (•—•—•). P = polypeptide P, mol wt. 83 500; NP = nucleoprotein, mol wt. 60 000; $HA_1$, $HA_2$ = haemagglutinin subunits 1 and 2, mol wt. 49 000 and 32 000; NA = neuraminidase, mol wt. 45 000; M = polypeptide M, mol wt. 26 500 [8].

techniques that among the proteins synthesized there is one which is related antigenically to the ribonucleoprotein-A (RNP-A).

We therefore conclude that at least some of the influenza genes are encoded in the virion type-RNA and not in the complementary strand.

The described approach opens a direct way to study the coding properties of influenza RNA in more detail as will be shown in a further communication on this subject [9].

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