Synthesis of Influenza Virus Polypeptides in Cells Resistant to Alpha-Amanitin: Evidence for the Involvement of Cellular RNA Polymerase II in Virus Replication

ROBERT A. LAMB* AND PURNELL W. CHOPPIN
The Rockefeller University, New York, New York 10021

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Influenza virus polypeptides were not synthesized in wild-type CHO-S-infected cells in the presence of α-amaminitin, but were synthesized in CHO-Amal cells, a mutant cell line whose DNA-dependent RNA polymerase II is specifically resistant to this drug, indicating that this cellular enzyme is involved in influenza virus replication. The results of experiments designed to detect viral polypeptides synthesized from primary transcripts suggest that the synthesis of a cellular RNA species by RNA polymerase II is required for primary transcription of the influenza virus genome.

In 1954, influenza virus was shown to be inhibited by 5,6-dichloro-1,β-D-ribofuranosyl-benzimidazole (DRB). It was suggested at that time that DRB interfered with nucleic acid metabolism (26, 28), and recently it has been shown that DRB is an inhibitor of heterogeneous nuclear and mRNA synthesis (25, 27). Subsequently, it was found that the replication of influenza virus is sensitive in its early stages to several inhibitors of DNA function, e.g., actinomycin D, mitomycin C, and UV light (1, 3, 16, 22). It has been proposed that participation of host cell DNA is needed for the replication of influenza virus (2) or, alternatively, that actinomycin D and other drugs that react with DNA inhibit influenza virus replication nonspecifically by degrading the input virion RNA molecules in the cell nucleus (9, 22) or by disorganizing the nucleolus (7). It has also been found that actinomycin D inhibits influenza virus complementary RNA synthesis at times late in infection (19, 24) and inhibits primary transcription in vivo but does not affect in vitro transcription by the virion-associated enzyme (4, 18). The precise mechanism for these effects of actinomycin D on influenza virus is not yet clear.

Further evidence suggesting that a function specified by host cell DNA is needed for influenza virus replication was provided by the finding that α-amaminitin, an inhibitor both in vivo and in vitro of DNA-dependent RNA polymerase II (6, 8, 10, 11, 17), which is involved in mRNA synthesis (20, 21), also inhibited the replication of influenza virus when added during the first 2 h of infection (15, 23). However, α-amaminitin did not inhibit in vitro transcription by the virion enzyme (18). Although the available evidence suggested that the inhibition of influenza virus by α-amaminitin and the other drugs was due to the inhibition of the synthesis of a cellular RNA species that was required for virus replication, the possibility had not been excluded that the effect on virus replication was due to a unique sensitivity of a virus-specific step in replication or to secondary effects on the cells.

Recently, Chinese hamster ovary cells that are resistant to α-amaminitin (5, 13) have been isolated, and it has been shown that the purified RNA polymerase II of these cells is insensitive to the drug (14). These cells provided a means of investigating directly whether the action of α-amaminitin on influenza virus replication is due to an effect on cellular RNA polymerase II, to a peculiar sensitivity of influenza virus replication to the inhibitor, or to a secondary effect on the cells or virus, as has been proposed for actinomycin D (7, 9, 22). We therefore examined the synthesis of influenza virus polypeptides in the α-amaminitin-resistant (CHO-Amal) and wild-type (CHO-S) Chinese hamster ovary cells.

CHO-S and CHO-Amal cells were grown in reinforced Eagle medium and 10% fetal calf serum, supplemented with nonessential amino acids, as described previously (12). Cells were infected with WSN influenza A virus, labeled with [35S]methionine for 30 min at various times after infection, and processed for gel electrophoresis and autoradiography as described previously (12). α-Amanitin (20 μg/ml) was added to cells 30 min before infection, and the inoculum and medium also contained the drug.
All of the known influenza virus polypeptides were synthesized in CHO-S cells, but in the presence of α-amanitin no viral polypeptides were detected up to 6 h after infection (Fig. 1). In contrast to these results in CHO-S cells, in the drug-resistant CHO-Amal cells, all of the viral polypeptides were synthesized in the presence of α-amanitin as well as in its absence, and there were no detectable differences in the levels of synthesis of the viral polypeptides in treated versus untreated cells. Similar results have been obtained by L. L. R. Spooner and R. D. Barry [Nature (London), in press].

This demonstration that influenza virus polypeptides are synthesized in the presence of α-amanitin in a mutant cell line whose RNA polymerase II is specifically resistant to the drug (14), but not in the wild-type cells, indicates that the inhibition of virus replication by the drug is due to inhibition of its action on the cellular enzyme. If the effect of the drug were on a virus-specific step in RNA or protein synthesis, no polypeptides would have been seen in the α-amanitin-resistant cells. Similarly, a nonspecific effect of the drug on the cells also appears to be eliminated. Thus, these results provide strong evidence for the previous suggestion (15, 29) that a host cell RNA species is required for influenza virus replication.

From previous studies in this laboratory using several other cell types (12), it would be expected that if primary transcription of the viral genome RNA had occurred in the CHO-S cells in the presence of α-amanitin, viral polypeptides translated from these mRNA's would have been detected under the labeling conditions used. This is demonstrated in both CHO-S cells and chicken embryo fibroblasts (CEF) in Fig. 2. By a procedure described previously (12), CHO-S and CEF cells were treated with cycloheximide (100 μg/ml), which permits primary transcription but inhibits replication and secondary transcription (4). The drug was present for 30 min before infection, during the adsorption period, and for 4 h after infection; then it was washed out, and the cells were labeled with

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**Fig. 1.** Synthesis of polypeptides in influenza virus-infected CHO-Amal and CHO-S cells in the presence (+) or (−) of α-amanitin. Cells were infected with a multiplicity of ~100 PFU/cell, and at the times indicated after infection were labeled for 30 min with [35S]methionine and subjected to electrophoresis on a 13% gel as described previously (12). CHO-Amal cells were labeled with 15 μCi and CHO-S cells were labeled with 20 μCi of [35S]methionine per ml.
[35S]methionine for 15 min at the times indicated after removal of the drug. Throughout the experiment, α-amanitin (20 μg/ml) added to cells 30 min before infection was present. In the absence of α-amanitin, polypeptides P₁, P₂, P₃, NP, M, and NS could be detected in both cell types immediately after removal of the cycloheximide block, and, in addition, polypeptide HA was detected immediately in CEF cells. Thus these polypeptides were translated from primary transcripts synthesized in the presence of cycloheximide. In contrast, in the α-amanitin-treated cells there was no viral polypeptide synthesis in either CHO-S or CEF cells after the release of the cycloheximide blocks. These results thus provide evidence that α-amanitin inhibits primary transcription of the influenza genome, and, coupled with the above results with the drug-resistant CHO-Amal cells, they suggest that a product of RNA polymerase II is required for primary transcription, which the available evidence suggested occurs in the nucleus (29).

The quantity of the HA and M polypeptides synthesized in CHO-S cells immediately after removal of cycloheximide was significantly less than that seen in CEF cells at that time. However, by 3 h later, the quantities of M and NS in both cell types were roughly equivalent. Thus, the host cell may affect the level of primary transcription of influenza virus RNA species or the efficiency of translation of some mRNA's.

Fig. 2. Synthesis of polypeptides in influenza virus-infected CHO-S and CEF cells after inhibition of protein synthesis for 4 h in the presence or absence of α-amanitin. CHO-S and CEF cells were treated for 30 min before infection with cycloheximide (100 μg/ml) during the adsorption period and for 4 h after infection, and then the drug was removed. α-Amanitin (20 μg/ml) was added to one group of cells 30 min before infection and was present throughout the experiment. After removal of cycloheximide, the cells were labeled with [35S]methionine (20 μCi/ml) for 15 min at the times indicated and subjected to electrophoresis on a 13% gel.
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LITERATURE CITED


